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SEARCH REQUEST FORM

50172

Requestor's Name: J. Schoenleyun

Serial Number: 09/509910

Date: 05/27/01 Phone: 30[illegible]12

10001
1003

Art Unit: 1644
CM1 10D16

Search Topic:

Please write a detailed statement of search topic. Describe specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples or relevent citations, authors, keywords, etc., if known. For sequences, please attach a copy of the sequence. You may include a copy of the broadest and/or most relevent claim(s).

Human IL-4 muteins which have reduced affinity and w/ altered specificity to the γ subunit of the IL-4 receptor and/or to IL-13Rα subunit & no IL-4 reception.

Muteins of IL4
Sebald, Walter

POINT OF CONTACT:
BARB O'BRYEN
TECH. INFORMATION SPECIALIST
STIC CM1 12C14 308-4291

=> fil capl; d que l22; d que l23; d que l25;d que l27; d que l46; s l22 or l23 or l25 or l27 or l46
FILE 'CAPLUS' ENTERED AT 14:30:35 ON 04 SEP 2001

FILE COVERS 1947 - 4 Sep 2001 VOL 135 ISS 11
FILE LAST UPDATED: 3 Sep 2001 (20010903/ED)

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The CA Lexicon is now available in the Controlled Term (/CT) field. Enter HELP LEXICON for full details.

L2	462	SEA FILE=CAPLUS ABB=ON	MUTEIN#
L4	12276	SEA FILE=CAPLUS ABB=ON	(HIL OR IL OR INTERLEUKIN#)(L)4/OBI OR HIL4/OBI OR IL4/OBI
L12	163779	SEA FILE=CAPLUS ABB=ON	MUTANT#/OBI OR MUTAT?/OBI
L15	12980	SEA FILE=CAPLUS ABB=ON	INTERLEUKIN(L)RECEPTOR#/CW
L16	223944	SEA FILE=CAPLUS ABB=ON	AFFINITY
L17	139225	SEA FILE=CAPLUS ABB=ON	SPECIFICITY
L18	235	SEA FILE=CAPLUS ABB=ON	L15(L)(L16 OR L17)
L22	3	SEA FILE=CAPLUS ABB=ON	L4 AND (L2 OR L12) AND L18

L2	462	SEA FILE=CAPLUS ABB=ON	MUTEIN#
L4	12276	SEA FILE=CAPLUS ABB=ON	(HIL OR IL OR INTERLEUKIN#)(L)4/OBI OR HIL4/OBI OR IL4/OBI
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L8	1193767	SEA FILE=CAPLUS ABB=ON	ALPHA
L10	63174	SEA FILE=CAPLUS ABB=ON	SUBUNIT#/OBI
L11	16774	SEA FILE=CAPLUS ABB=ON	(L5 OR L8)(L)L10
L12	163779	SEA FILE=CAPLUS ABB=ON	MUTANT#/OBI OR MUTAT?/OBI
L16	223944	SEA FILE=CAPLUS ABB=ON	AFFINITY
L17	139225	SEA FILE=CAPLUS ABB=ON	SPECIFICITY
L23	3	SEA FILE=CAPLUS ABB=ON	L4 AND (L2 OR L12) AND L11 AND (L16 OR

L17)

L2	462	SEA FILE=CAPLUS ABB=ON	MUTEIN#
L4	12276	SEA FILE=CAPLUS ABB=ON	(HIL OR IL OR INTERLEUKIN#)(L)4/OBI OR HIL4/OBI OR IL4/OBI
L12	163779	SEA FILE=CAPLUS ABB=ON	MUTANT#/OBI OR MUTAT?/OBI
L13	116	SEA FILE=CAPLUS ABB=ON	L4(L)(L2 OR L12)
L24	53400	SEA FILE=CAPLUS ABB=ON	MOLECULAR ASSOCIATION/CT
L25	4	SEA FILE=CAPLUS ABB=ON	L13 AND L24

L2	462	SEA FILE=CAPLUS ABB=ON	MUTEIN#
L4	12276	SEA FILE=CAPLUS ABB=ON	(HIL OR IL OR INTERLEUKIN#)(L)4/OBI OR HIL4/OBI OR IL4/OBI
L12	163779	SEA FILE=CAPLUS ABB=ON	MUTANT#/OBI OR MUTAT?/OBI
L13	116	SEA FILE=CAPLUS ABB=ON	L4(L)(L2 OR L12)
L26	60282	SEA FILE=CAPLUS ABB=ON	MOLECULAR STRUCTURE-BIOLOGICAL ACTIVITY RELATIONSHIP/CT
L27	8	SEA FILE=CAPLUS ABB=ON	L13 AND L26

L2	462	SEA FILE=CAPLUS ABB=ON	MUTEIN#
L4	12276	SEA FILE=CAPLUS ABB=ON	(HIL OR IL OR INTERLEUKIN#)(L)4/OBI OR HIL4/OBI OR IL4/OBI
L5	631920	SEA FILE=CAPLUS ABB=ON	GAMMA
L6	16	SEA FILE=CAPLUS ABB=ON	HIL(W)(13 OR 13R)
L7	7	SEA FILE=CAPLUS ABB=ON	HIL13?
L8	1193767	SEA FILE=CAPLUS ABB=ON	ALPHA
L12	163779	SEA FILE=CAPLUS ABB=ON	MUTANT#/OBI OR MUTAT?/OBI
L19	116	SEA FILE=CAPLUS ABB=ON	L4 (L)(L2 OR L12)
L29	93532	SEA FILE=CAPLUS ABB=ON	(ANTAGONIST# OR AGONIST#)/OBI
L31	387707	SEA FILE=CAPLUS ABB=ON	RECEPTOR#/OBI
L32	16	SEA FILE=CAPLUS ABB=ON	L19 AND L29 AND L31
L46	9	SEA FILE=CAPLUS ABB=ON	L32 AND (L5 OR L6 OR L7 OR L8)

L129 21 L22 OR L23 OR L25 OR L27 OR L46

=> fil medl; d que 158
FILE 'MEDLINE' ENTERED AT 14:30:47 ON 04 SEP 2001

FILE LAST UPDATED: 3 SEP 2001 (20010903/UP). FILE COVERS 1958 TO DATE.

On April 22, 2001, MEDLINE was reloaded. See HELP RLOAD for details.

MEDLINE now contains IN-PROCESS records. See HELP CONTENT for details.

MEDLINE is now updated 4 times per week. A new current-awareness alert frequency (EVERYUPDATE) is available. See HELP UPDATE for more information.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2001 vocabulary. Enter HELP THESAURUS for details.

The OLDMEDLINE file segment now contains data from 1958 through 1965. Enter HELP CONTENT for details.

Left, right, and simultaneous left and right truncation are available in the Basic Index. See HELP SFIELDS for details.

THIS FILE CONTAINS CAS REGISTRY NUMBERS FOR EASY AND ACCURATE SUBSTANCE IDENTIFICATION.

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L50        9641 SEA FILE=MEDLINE ABB=ON  INTERLEUKIN-4/CT
L51         665 SEA FILE=MEDLINE ABB=ON  RECEPTORS, INTERLEUKIN-4/CT
L53      254532 SEA FILE=MEDLINE ABB=ON  MUTATION+NT/CT
L54          43 SEA FILE=MEDLINE ABB=ON  L50 AND L51 AND L53
L55      505286 SEA FILE=MEDLINE ABB=ON  AFFINITY OR SPECIFICITY
L57      105343 SEA FILE=MEDLINE ABB=ON  SPECIES SPECIFICITY/CT
L58           9 SEA FILE=MEDLINE ABB=ON  L54 AND L55 NOT L57
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=> fil embase; d que l77; fil wpids; d que l85; fil biosis; d que l95; fil biotechno; d que l117;d que l122; s l117 or l122; fil biotechds; d que l128
FILE 'EMBASE' ENTERED AT 14:31:19 ON 04 SEP 2001

FILE COVERS 1974 TO 30 Aug 2001 (20010830/ED)

EMBASE has been reloaded. Enter HELP RLOAD for details.

This file contains CAS Registry Numbers for easy and accurate substance identification.

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L66       13597 SEA FILE=EMBASE ABB=ON  INTERLEUKIN 4/CT
L67         596 SEA FILE=EMBASE ABB=ON  INTERLEUKIN 4 RECEPTOR/CT OR INTERLEUKI
              N 4 RECEPTOR ALPHA/CT
L68      184925 SEA FILE=EMBASE ABB=ON  MUTATION+NT/CT
L70      293807 SEA FILE=EMBASE ABB=ON  AFFINITY OR SPECIFICITY
L72       19621 SEA FILE=EMBASE ABB=ON  AMINO ACID SUBSTITUTION/CT
L75        5707 SEA FILE=EMBASE ABB=ON  L66/MAJ
L77          10 SEA FILE=EMBASE ABB=ON  L75 AND (L68 OR L72) AND L67 AND L70
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FILE 'WPIDS' ENTERED AT 14:31:20 ON 04 SEP 2001

FILE LAST UPDATED: 31 AUG 2001 <20010831/UP>
MOST RECENT DERWENT UPDATE 200149 <200149/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

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L80       13282 SEA FILE=WPIDS ABB=ON  MUTEIN# OR MUTANT? OR MUTAT?
L81         553 SEA FILE=WPIDS ABB=ON  (IL OR HIL OR INTERLEUKIN)(W)4 OR IL4
                OR HIL4
L82       27882 SEA FILE=WPIDS ABB=ON  RECEPTOR#
L83       25083 SEA FILE=WPIDS ABB=ON  AFFINITY OR SPECIFICITY
L84        2629 SEA FILE=WPIDS ABB=ON  AMINO ACID# (3A)(REPLAC? OR SUBSTITUT?)

L85           9 SEA FILE=WPIDS ABB=ON  L81 AND L82 AND L83 AND (L80 OR L84)


FILE 'BIOSIS' ENTERED AT 14:31:20 ON 04 SEP 2001
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FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 29 August 2001 (20010829/ED)

The BIOSIS file has been reloaded.  Enter HELP RLOAD and HELP REINDEXING
for details.


L86       20767 SEA FILE=BIOSIS ABB=ON  (IL OR HIL OR INTERLEUKIN)(W)4 OR IL4
                OR HIL4
L87      611800 SEA FILE=BIOSIS ABB=ON  RECEPTOR#
L88      314454 SEA FILE=BIOSIS ABB=ON  AFFINITY OR SPECIFICITY
L89      329912 SEA FILE=BIOSIS ABB=ON  MUTEIN# OR MUTANT? OR MUTAT?
L90       14157 SEA FILE=BIOSIS ABB=ON  AMINO ACID# (3A)(REPLAC? OR SUBSTITUT?)

L92         123 SEA FILE=BIOSIS ABB=ON  L86(5A)(L89 OR L90)
L93          23 SEA FILE=BIOSIS ABB=ON  L92 AND L87(L)L88
L94      696775 SEA FILE=BIOSIS ABB=ON  GAMMA OR ALPHA
L95          12 SEA FILE=BIOSIS ABB=ON  L93 AND L94


FILE 'BIOTECHNO' ENTERED AT 14:31:21 ON 04 SEP 2001
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FILE LAST UPDATED: 28 AUG 2001      <20010828/UP>
FILE COVERS 1980 TO DATE.

>>>  SIMULTANEOUS LEFT AND RIGHT TRUNCATION AVAILABLE IN
     /CT AND BASIC INDEX  <<<


L96       10899 SEA FILE=BIOTECHNO ABB=ON  (IL OR HIL OR INTERLEUKIN)(W)4 OR
                IL4 OR HIL4
L99      173492 SEA FILE=BIOTECHNO ABB=ON  MUTEIN# OR MUTANT? OR MUTAT?
L100      18885 SEA FILE=BIOTECHNO ABB=ON  AMINO ACID# (3A)(REPLAC? OR
                SUBSTITUT?)
L105       7990 SEA FILE=BIOTECHNO ABB=ON  INTERLEUKIN 4/CT
L106        415 SEA FILE=BIOTECHNO ABB=ON  INTERLEUKIN 4 RECEPTOR/CT
L107       4928 SEA FILE=BIOTECHNO ABB=ON  RECEPTOR AFFINITY/CT
L109         96 SEA FILE=BIOTECHNO ABB=ON  L96(5A)(L99 OR L100)
L117          4 SEA FILE=BIOTECHNO ABB=ON  L105 AND L106 AND L107 AND L109
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L96       10899 SEA FILE=BIOTECHNO ABB=ON  (IL OR HIL OR INTERLEUKIN)(W)4 OR
                IL4 OR HIL4
L98      134110 SEA FILE=BIOTECHNO ABB=ON  AFFINITY OR SPECIFICITY
L99      173492 SEA FILE=BIOTECHNO ABB=ON  MUTEIN# OR MUTANT? OR MUTAT?
L100      18885 SEA FILE=BIOTECHNO ABB=ON  AMINO ACID# (3A)(REPLAC? OR
                SUBSTITUT?)
L101     192080 SEA FILE=BIOTECHNO ABB=ON  ALPHA OR GAMMA
L105       7990 SEA FILE=BIOTECHNO ABB=ON  INTERLEUKIN 4/CT
L106        415 SEA FILE=BIOTECHNO ABB=ON  INTERLEUKIN 4 RECEPTOR/CT
L109         96 SEA FILE=BIOTECHNO ABB=ON  L96(5A)(L99 OR L100)
L120      65671 SEA FILE=BIOTECHNO ABB=ON  MUTATION/CW
L121      14867 SEA FILE=BIOTECHNO ABB=ON  AMINO ACID SUBSTITUTION/CT
L122          3 SEA FILE=BIOTECHNO ABB=ON  L109 AND L105 AND L106 AND L101 AND
                L98 AND (L120 OR L121)


L130          5 L117 OR L122

FILE 'BIOTECHDS' ENTERED AT 14:31:22 ON 04 SEP 2001
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L123        478 SEA FILE=BIOTECHDS ABB=ON  (IL OR HIL OR INTERLEUKIN)(W)4 OR
                IL4 OR HIL4
L124       8848 SEA FILE=BIOTECHDS ABB=ON  RECEPTOR#
L125      23551 SEA FILE=BIOTECHDS ABB=ON  MUTEIN# OR MUTANT? OR MUTATION?
L126       1439 SEA FILE=BIOTECHDS ABB=ON  AMINO ACID(3A)(SUBSTITUT? OR
                REPLAC?)
L127      15625 SEA FILE=BIOTECHDS ABB=ON  AFFINITY OR SPECIFICITY
L128          4 SEA FILE=BIOTECHDS ABB=ON  L123 AND L124 AND (L125 OR L126)
                AND L127


=> dup rem 158,1129,195,1130,1128,177,185
FILE 'MEDLINE' ENTERED AT 14:32:45 ON 04 SEP 2001

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FILE 'WPIDS' ENTERED AT 14:32:45 ON 04 SEP 2001
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PROCESSING COMPLETED FOR L129
PROCESSING COMPLETED FOR L95
PROCESSING COMPLETED FOR L130
PROCESSING COMPLETED FOR L128
PROCESSING COMPLETED FOR L77
PROCESSING COMPLETED FOR L85
L131 50 DUP REM L58 L129 L95 L130 L128 L77 L85 (20 DUPLICATES REMOVED)
ANSWERS '1-9' FROM FILE MEDLINE
ANSWERS '10-29' FROM FILE CAPLUS
ANSWERS '30-37' FROM FILE BIOSIS
ANSWERS '38-39' FROM FILE BIOTECHNO
ANSWERS '40-41' FROM FILE BIOTECHDS
ANSWERS '42-46' FROM FILE EMBASE
ANSWERS '47-50' FROM FILE WPIDS

=> d ibib ab 1-50; fil hom

L131 ANSWER 1 OF 50 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 97203116 MEDLINE
DOCUMENT NUMBER: 97203116 PubMed ID: 9050834
TITLE: A mixed-charge pair in human interleukin 4 dominates high-**affinity** interaction with the receptor alpha chain.
AUTHOR: Wang Y; Shen B J; Sebald W
CORPORATE SOURCE: Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum) der Universitat, Physiologische Chemie II, Wurzburg, Germany.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Mar 4) 94 (5) 1657-62. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970422
Last Updated on STN: 19980206
Entered Medline: 19970407

AB Human interleukin 4 (IL-4) binds to its cellular receptor with a Kd in the subnanomolar range, similar to many other 4-helix-bundle proteins interacting with members of the hematopoietin (cytokine) receptor superfamily. In the IL-4 system this interaction is predominantly determined by the extracellular domain (IL4-BP) of the receptor alpha chain (Kd approximately 150 pM). Now a high-resolution mutational and kinetic analysis has revealed that the high-**affinity** binding of IL-4 originates from a continuous patch of a few mostly polar or charged amino acid side chains located on helices A and C. The binding epitope comprises (i) a set of side chains determining the dissociation rate (k(off)) and (ii) a partially overlapping set determining the association rate constant (k(on)) of the IL-4/IL4-BP complex. The k(off) epitope is assembled from two juxtaposed main determinants (Glu-9 and Arg-88) surrounded by five side chains (Ile-5, Thr-13, Arg-53, Asn-89, and Trp-91) of lower importance. The cumulative increase in k(off) after alanine substitution is 10(5)-fold for the central mixed-charge pair and 3 x 10(3)-fold for the satellites. The k(on) epitope is formed by five positively charged residues on helix C (Lys-77, Arg-81, Lys-84, Arg-85, and Arg-88) and two neighboring residues on helix A (Glu-9 and Thr-13). The cumulative loss in k(on) of the alanine variants is only about 10-fold. These results provide the basis for an understanding of molecular

recognition in cytokine receptor complexes and for an IL-4 antagonist design.

L131 ANSWER 2 OF 50 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 96390888 MEDLINE
DOCUMENT NUMBER: 96390888 PubMed ID: 8797861
TITLE: Global and local determinants for the kinetics of interleukin-4/interleukin-4 receptor alpha chain interaction. A biosensor study employing recombinant interleukin-4-binding protein.
AUTHOR: Shen B J; Hage T; Sebald W
CORPORATE SOURCE: Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum) Universitat Physiologische Chemie II, Wurzburg, Germany.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1996 Aug 15) 240 (1) 252-61.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19990129
Entered Medline: 19961104

AB An engineered interleukin-4-binding protein (IL4-BP) representing the extracellular domain of the human interleukin-4 (IL-4) receptor alpha chain was expressed in Sf9 cells. The purified IL4-BP was immobilized via a single biotinylated SH group near the carboxyl end to a biosensor matrix and analysed in real time for interaction with IL-4 and IL-4 variants. IL-4 was bound to IL4-BP at a molar ratio of approximately 1:1. The association and dissociation at pH 7.4 and 150 mM NaCl had rate constants of 1.9 +/- 0.3 x 10(7) M-1 s-1 and 2 +/- 1 x 10(-3) s-1, respectively. Glycosylation and engineered amino acid substitutions of IL4-BP did not alter the kinetic constants as shown by a parallel analysis of IL4-BP variants produced in Escherichia coli or Chinese hamster ovary cells. The rate of association was only slightly affected in binding-deficient variants [E9Q]IL-4 and [R88Q]IL-4 and by acidic pH down to values of 4.5, but it was reduced up to fivefold at higher ionic strength. The rate of dissociation was increased 70-fold and 150-fold with the IL-4 variants and fivefold at an acidic pH of 4.5, but it was not affected by high ionic strength. Temperatures between 6 degrees C and 37 degrees C yielded similar rates of IL-4 dissociation and only a marginally reduced rate of IL-4 association at 6 degrees C. These results indicate that the high-**affinity** binding of IL-4 to its receptor (Kd approximately 100 pM) is mainly the result of an unusually high association rate. The IL-4/IL4-BP interaction appears to be dominated by charge effects. The exceedingly high rate of IL-4/IL4-BP association is augmented by the overall electrostatic potentials of both proteins (electrostatic steering). Localized charges and the formation of ion pairs may control the rate of complex dissociation.

L131 ANSWER 3 OF 50 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 96055894 MEDLINE
DOCUMENT NUMBER: 96055894 PubMed ID: 7575356
TITLE: Antagonistic mutant proteins of interleukin-4.
AUTHOR: Duschl A; Muller T; Sebald W
CORPORATE SOURCE: Theodor-Boveri-Institut fur Biowissenschaften, Universitat Wurzburg, Germany.
SOURCE: BEHRING INSTITUTE MITTEILUNGEN, (1995 Jun) (96) 87-94.
Ref: 27
Journal code: 9KI; 0367532. ISSN: 0301-0457.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 19980206
Entered Medline: 19951122

AB Interleukin-4 is a major regulator of the immune system, directing e.g. induction of a TH2 phenotype in T-cells, activation of B-cells and synthesis of IgE type antibodies, which are associated with allergic responses. Site-directed mutagenesis has revealed two sites important for receptor interaction on IL-4: site I mediates binding to the IL-4 receptor alpha subunit, and site II is involved in signal transduction through the receptor complex. Specific mutations in site II produced a series of ligands which bound to the receptor with high **affinity**, but had little or no agonistic activity and inhibited effects of wild type IL-4. The closely related cytokine IL-13, also a mediator of allergic processes, is antagonized as well. Antagonistic site II mutants of human IL-4 are therefore effective inhibitors with therapeutic potential for IL-4 associated diseases like type I hypersensitivity and asthma.

L131 ANSWER 4 OF 50 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 93327755 MEDLINE
DOCUMENT NUMBER: 93327755 PubMed ID: 8101483
TITLE: Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction.
AUTHOR: Zurawski S M; Vega F Jr; Huyghe B; Zurawski G
CORPORATE SOURCE: Department of Molecular Biology, DNAX Research Institute for Molecular and Cellular Biology, Palo Alto, CA 94304-1104.
SOURCE: EMBO JOURNAL, (1993 Jul) 12 (7) 2663-70.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199308
ENTRY DATE: Entered STN: 19930903
Last Updated on STN: 19980206
Entered Medline: 19930820

AB Interleukin-4 (IL-4) and interleukin-13 (IL-13) are two cytokines that are secreted by activated T cells and have similar effects on monocytes and B cells. We describe a mutant form of human interleukin-4 (hIL-4) that competitively antagonizes both hIL-4 and human interleukin-13 (hIL-13). The amino acid sequences of IL-4 and IL-13 are approximately 30% homologous and circular dichroism (CD) spectroscopy shows that both proteins have a highly alpha-helical structure. IL-13 competitively inhibited binding of hIL-4 to functional human IL-4 receptors (called hIL-4R) expressed on a cell line which responds to both hIL-4 and IL-13. Binding of hIL-4 to an hIL-4 responsive cell line that does not respond to IL-13, and binding of hIL-4 to cloned IL-4R ligand binding protein expressed on heterologous cells, were not inhibited by IL-13. hIL-4 bound with approximately 100-fold lower **affinity** to the IL-4R ligand binding protein than to functional IL-4R. The mutant hIL-4 antagonist protein bound to both IL-4R types with the lower **affinity**. The above results demonstrate that IL-4 and IL-13 share a receptor component that is important for signal transduction. In addition, our data establish

that IL-4R is a complex of at least two components one of which is a novel **affinity** converting subunit that is critical for cellular signal transduction.

L131 ANSWER 5 OF 50 MEDLINE
ACCESSION NUMBER: 2001118890 MEDLINE
DOCUMENT NUMBER: 21070640 PubMed ID: 11202474
TITLE: Polymorphisms in candidate asthma genes.
AUTHOR: Nanavaty U; Goldstein A D; Levine S J
CORPORATE SOURCE: Critical Care Medicine Department, National Institutes of Health, Bethesda, Maryland, USA.
SOURCE: AMERICAN JOURNAL OF THE MEDICAL SCIENCES, (2001 Jan) 321 (1) 11-6. Ref: 32
Journal code: 3L2. ISSN: 0002-9629.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215

AB The triad of reversible airway obstruction, bronchial hyperresponsiveness, and airway inflammation characterizes asthma. The etiology of asthma is complex and involves the interaction of multiple genetic foci and a variety of environmental factors, such as protein allergens, chemical sensitizers, and viral or bacterial proteins. Candidate asthma genes have been identified that may be linked or associated with the asthmatic phenotype. Potential candidate asthma genes include cytokine genes, receptor genes, transcription factors, immune recognition genes, and genes regulating lipid mediator generation. Although polymorphisms within either the promoter or coding region of individual asthma candidate genes have been identified, the association between these genetic polymorphisms and the asthmatic phenotype remains incompletely defined. Furthermore, genetic polymorphisms mediating the asthmatic phenotype are rarely identified in individual patients. This manuscript reviews several of the specific mutations and polymorphisms that have been identified in candidate asthma genes, such as the high **affinity** IgE receptor, the beta2-adrenergic receptor, the interleukin-4 promoter and receptor, the tumor necrosis factor gene, and the 5-lipoxygenase promoter.

L131 ANSWER 6 OF 50 MEDLINE
ACCESSION NUMBER: 1999171151 MEDLINE
DOCUMENT NUMBER: 99171151 PubMed ID: 10071757
TITLE: Binding of interleukin-13 and interleukin-4 to the interleukin (IL)-4/IL-13 receptor of human synovial fibroblasts.
AUTHOR: Lutz R A; Feng N; Moser R
CORPORATE SOURCE: Institute of Clinical Chemistry, University Hospital, Zurich.
SOURCE: JOURNAL OF RECEPTOR AND SIGNAL TRANSDUCTION RESEARCH, (1999 Jan-Jul) 19 (1-4) 181-90.
Journal code: CCU; 9509432. ISSN: 1079-9893.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990628

Last Updated on STN: 19990628
Entered Medline: 19990615

AB Synovial fibroblasts expressed transcripts for IL-4R alpha, and IL-13R alpha 1 and IL-13R alpha 2. Using weighted nonlinear computer modeling of the data from equilibrium binding studies, a 2 bindings sites model fitted the data best. After occupation of the shared high **affinity** receptors by the non-signaling, double mutant IL-4(121)R-->D, 124Y-->D (RY-IL-4) the high **affinity** binding of IL-13 could be abolished. A 2 binding site model still could be fitted, however the improvement in fit over a onesite model was not statistically significant. Using **affinity** spectra, at least 2 binding sites are apparent. After treatment with RY-IL-4, some of the high **affinity** binding was abolished, however not completely. A correlation between the number of binding sites and the **affinity** is apparent, which seriously casts doubt on the classical evaluation of binding isotherms, where the parameters are assumed to be independent. In a previous study we suggested that the large number of IL-13R alpha 2 monomers are silent receptors, likely representing a decoy target for IL-13. The high **affinity** binding therefore most likely represents the binding to the heterodimer consisting of IL-4R alpha and IL-13R alpha 1 or IL-13R alpha 2. The low **affinity** binding may represent the IL-13R alpha 2.

L131 ANSWER 7 OF 50 MEDLINE
ACCESSION NUMBER: 96089927 MEDLINE
DOCUMENT NUMBER: 96089927 PubMed ID: 7590938
TITLE: The critical region in the cytoplasmic domain of human IL-4 receptor for induction of IgE synthesis.
AUTHOR: Schultz C; Izuhara K; Coffman R; Harada N
CORPORATE SOURCE: Department of Immunology, DNAX Research Institute, Palo Alto, CA 94304-1104, USA.
SOURCE: IMMUNOLOGY LETTERS, (1995 Jun) 46 (3) 215-9.
Journal code: GIH; 7910006. ISSN: 0165-2478.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19980206
Entered Medline: 19951228

AB To examine the region critical for differentiation in the human IL-4 receptor (hIL-4R), we transfected the Abelson murine leukemia virus (A-MuLV)-transformed murine pre-B cell line A20 with plasmid DNA encoding the hIL-4R. Transfectants expressed high **affinity** hIL-4Rs on the cell surface. Treatment with LPS and hIL-4 induced germline C epsilon transcripts in hIL-4R expressing A20 cells. Several hIL-4R mutant plasmids were then transfected into A20 cells and the transfectants were examined for hIL-4R expression and the ability to induce germline C epsilon transcripts upon stimulation with LPS and hIL-4. Although all A20 transfectants tested expressed the high-**affinity** hIL-4R, A20 transfectants expressing the mutant hIL-4R, which contains only 8 amino acids in the cytoplasmic domain, did not respond to LPS and hIL-4 with germline C epsilon transcripts. In addition, A20 transfectants expressing an internally deleted hIL-4R, in which the deleted region has been identified as the critical region for growth signal transduction in the previous study, failed to induce germline C epsilon transcripts with LPS and hIL-4. These results indicate that the critical region for the differentiation signal in the hIL-4R is identical to that for the growth signal, suggesting that IL-4 may share, at least partly, a common signal pathway for both growth and differentiation.

L131 ANSWER 8 OF 50 MEDLINE
ACCESSION NUMBER: 93054586 MEDLINE
DOCUMENT NUMBER: 93054586 PubMed ID: 1429625
TITLE: Identification of an essential region for growth signal transduction in the cytoplasmic domain of the human interleukin-4 receptor.
AUTHOR: Harada N; Yang G; Miyajima A; Howard M
CORPORATE SOURCE: Department of Immunology, DNAX Research Institute of Molecular and Cellular Biology, Inc., Palo Alto, California 94304-1104.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Nov 15) 267 (32) 22752-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19980206
Entered Medline: 19921216

AB Interleukin-4 (IL-4) is a pleiotropic lymphokine which plays an important role in the immune system by regulating proliferation and differentiation of a wide variety of lymphoid and myeloid cells. These biological effects are manifested via binding of IL-4 to specific membrane-associated high **affinity** receptors. While the IL-4 receptor (IL-4R) cDNA expresses high **affinity** binding sites when transfected in COS7 cells, its intracellular domain lacks consensus motifs for known signal transducing molecules such as a tyrosine kinase. In this study, we use a DNA deletion approach to explore the mechanism of signal transduction utilized by the human IL-4R cDNA expressed in a murine pro-B cell line, Ba/F3 cells. Using this system, we have identified the critical region of the cytoplasmic domain of human IL-4R for human IL-4-induced transduction of a growth signal in these cells. Our data indicate that the critical region for signal transduction is located between amino acid residues 433-473 numbering from the carboxyl terminus. This region is highly conserved between mouse and human IL-4R but lacks homology with other cytokine receptors. Our studies additionally demonstrate that the cytoplasmic domain is not essential for forming high **affinity** IL-4-binding sites nor for ligand internalization.

L131 ANSWER 9 OF 50 MEDLINE
ACCESSION NUMBER: 93028322 MEDLINE
DOCUMENT NUMBER: 93028322 PubMed ID: 1409544
TITLE: Phe496 and Leu497 are essential for receptor binding and cytotoxic action of the murine interleukin-4 receptor targeted fusion toxin DAB389-mIL-4.
AUTHOR: Lakkis F; Landgraf B; Wen Z; Strom T B; Murphy J R
CORPORATE SOURCE: Evans Department of Clinical Research, University Hospital, Boston, MA 02118.
CONTRACT NUMBER: U01 CA-48626 (NCI)
SOURCE: PROTEIN ENGINEERING, (1992 Apr) 5 (3) 241-8.
Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19980206
Entered Medline: 19921103

AB DAB389-mIL-4 is a murine interleukin-4 (mIL-4) diphtheria toxin-related fusion protein which has been shown to be selectively toxic to cells expressing the mIL-4 receptor. In this report, we have used site-directed and in-frame deletion mutagenesis to study the role of the putative C-terminal alpha-helix (helix E) of the mIL-4 component of DAB389-mIL-4 in the intoxication process. We demonstrate that deletion of the C-terminal 15 amino acids of the fusion toxin leads to loss of cytotoxicity. The substitution of Phe496 with either Pro, Ala or Tyr, results in a greater than 20-fold decrease in cytotoxic activity of the respective mutant fusion toxins. In addition, substitution of Leu497 with either Ala or Glu results in a similar loss of cytotoxic activity. All of these mutant forms of the mIL-4 fusion toxin demonstrate a significant decrease in binding **affinity** (Ki) to the mIL-4 receptor in a competitive radioligand binding assay. In marked contrast, however, the substitution of Asp495 with Asn results in a 4-fold increase in cytotoxic potency and binding **affinity** to mIL-4 receptor bearing cells in vitro.

L131 ANSWER 10 OF 50 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 2000:127531 CAPLUS
DOCUMENT NUMBER: 132:179594
TITLE: High-affinity **interleukin-4** **muteins**
INVENTOR(S): Greve, Jeffrey M.; Shanafelt, Armen B.; Roczniak, Steven
PATENT ASSIGNEE(S): Bayer Corporation, USA
SOURCE: U.S., 23 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6028176	A	20000222	US 1997-897020	19970718
PRIORITY APPLN. INFO.:			US 1996-22537	19960719

AB This invention is directed to recombinant human IL-4 **muteins** numbered in accordance with wild-type IL-4 wherein the **muteins** comprise at least one amino acid substitution selected from the group consisting of substitutions at positions 13, 16, 81 and 89 of the wild-type IL-4, whereby the **mutein** binds to the IL-4R. **alpha**. receptor with at least greater affinity than native IL-4. The invention is further directed to recombinant human IL-4 antagonist **muteins** numbered in accordance with wild-type IL-4 wherein the **muteins** comprise substitutions R121D and Y124D in the D-helix of said wild-type IL-4; and at least one amino acid substitution selected from the group consisting of substitutions at positions 13, 16, 81 and 89 of said wild-type IL-4, whereby the **mutein** binds to the IL-4R. **alpha**. receptor with at least greater affinity than native IL-4. The invention is also directed to pharmaceutical compns. comprising individual **muteins** in combination with pharmaceutically acceptable carriers. IL-4 **mutein** antagonist is useful for treating autoimmune diseases, e.g. rheumatoid arthritis, multiple sclerosis, and IDDM.

REFERENCE COUNT: 29
REFERENCE(S): (1) Anon; EP 0230107 1987 CAPLUS
(2) Anon; WO 8702990 1987 CAPLUS
(3) Anon; WO 8804667 1988 CAPLUS
(4) Anon; WO 9221029 1992 CAPLUS
(5) Anon; WO 9321308 1993 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

Searched by Barb O'Bryen, STIC 308-4291

L131 ANSWER 11 OF 50 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
ACCESSION NUMBER: 1999:279768 CAPLUS
DOCUMENT NUMBER: 130:280863
TITLE: **Interleukin 4** derivatives showing low-**affinity** and short-term interaction with the common .gamma. chain of the **interleukin** receptors
INVENTOR(S): Sebald, Walter
PATENT ASSIGNEE(S): Bayer A.-G., Germany
SOURCE: Eur. Pat. Appl., 13 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 911401	A1	19990428	EP 1997-118219	19971021
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
WO 9920765	A1	19990429	WO 1998-EP6448	19981012
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9897495	A1	19990510	AU 1998-97495	19981012
EP 1023446	A1	20000802	EP 1998-951511	19981012
R: DE, ES, FR, GB, IT				
ZA 9809540	A	19990422	ZA 1998-9540	19981020

PRIORITY APPLN. INFO.: EP 1997-118219 A 19971021
WO 1998-EP6448 W 19981012

AB Human IL-4 (IL-4), one of the small 4-helix-bundle cytokines, uses the specific IL-4 receptor a chain together with the common .gamma. chain (.gamma.c) for transmembrane signaling. The ligand-binding properties of .gamma.c, which are presently poorly understood, were analyzed by biosensor techniques employing recombinant ectodomains gamex (.gamma.c) and IL4-BP (a) of the receptor chains. The formation and decay of a ternary complex between solute gamex and IL-4 liganded IL4-BP could be established to exhibit a low **affinity** (Kd = 3.mu.M) as well as a short half life t1/2 = 7s. This binding **affinity** resulted largely from the interaction of gamex with IL-4 and not from a direct contact of IL4-BP and gamex, since the binary complex between solute gamex and immobilized IL-4 showed an only 50-fold greater Kd of 150 .mu.M. The IL-4 residues involved in gamex binding were identified by means of an alanine-scanning mutational approach. A functional gamex binding IL-4 epitope in which residues isoleucine-11, asparagine-15, and tyrosine-124 play significant roles is proposed. Even IL-4 variants which bind gamex 300-fold weaker than IL-4 with a dissocn. half life t1/2 of less than 1s, retained a substantial T-cell proliferative activity. These findings suggest that low **affinity** .gamma.c binding and short half lives of the heterodimeric a/.gamma.c receptor complex are sufficient for initiating IL-4 dependent signal transduction.

REFERENCE COUNT: 6
REFERENCE(S): (2) Duschl, A; EUROPEAN JOURNAL OF CYTOKINE NETWORK 1996, V7(1), P37 CAPLUS

(3) Kruse, N; EMBO JOURNAL 1993, V12(13), P5121 CAPLUS
(4) Matthews, D; EUROPEAN JOURNAL OF IMMUNOLOGY 1997, V27, P116 MEDLINE
(5) Ramanathan, L; BIOCHEMISTRY 1993, V32, P3549 CAPLUS
(6) Wang, Y; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA 1997, V94, P1657 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L131 ANSWER 12 OF 50 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
ACCESSION NUMBER: 1998:6293 CAPLUS
DOCUMENT NUMBER: 128:113915
TITLE: The association of atopy with a gain-of-function **mutation** in the **.alpha.** **subunit** of the **interleukin-4** receptor
AUTHOR(S): Khurana Hershey, Gurjit K.; Friedrich, Michal F.; Esswein, Laura A.; Thomas, Matthew L.; Chatila, Talal A.
CORPORATE SOURCE: Dep. Pediatrics, Washington Univ. School Medicine, St. Louis, MO, USA
SOURCE: N. Engl. J. Med. (1997), 337(24), 1720-1725
CODEN: NEJMAG; ISSN: 0028-4793
PUBLISHER: Massachusetts Medical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Using single-strand conformation polymorphism anal. and DNA sequencing, we searched for mutations in the .alpha. subunit of the interleukin-4 receptor that would predispose persons to atopy. We examd. the prevalence of the alleles among patients with allergic inflammatory disorders and among 50 prospectively recruited adults. Subjects with atopy were identified on the basis of an elevated serum IgE level (.gtoreq.95 IU per mL) or a pos. radio-immunosorbent test in response to std. inhalant allergens. The signaling function of mutant interleukin-4 receptor .alpha. was examd. by flow cytometry, binding assays, and immunoblotting. A novel interleukin-4 receptor .alpha. allele was identified in which guanine was substituted for adenine at nucleotide 1902, causing a change from glutamine to arginine at position 576 (R576) in the cytoplasmic domain of the interleukin-4 receptor .alpha. protein. The R576 allele was common among patients with allergic inflammatory disorders (found in 3 of 3 patients with the hyper-IgE syndrome and 4 of 7 patients with severe atopic dermatitis) and among the 50 prospectively recruited adults (found in 13 of 20 subjects with atopy and 5 of 30 without atopy; P=0.001; relative risk of atopy among those with a mutant allele, 9.3). The R576 allele was assocd. with higher levels of expression of CD23 by interleukin-4 than the wild-type allele. This enhanced signaling was assocd. with a change in the binding **specificity** of the adjacent tyrosine residue at position 575 to signal-transducing mols. Thus, the R576 allele of interleukin-4 receptor .alpha. is strongly assocd. with atopy. This mutation may predispose persons to allergic diseases by altering the signaling function of the receptor.

L131 ANSWER 13 OF 50 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 9
ACCESSION NUMBER: 1997:56394 CAPLUS
DOCUMENT NUMBER: 126:102947
TITLE: A murine **interleukin-4** -antagonistic **mutant** protein completely inhibits **interleukin-4**-induced cell proliferation, differentiation, and signal transduction
AUTHOR(S): Grunewald, Susanne M.; Kunzmann, Steffen; Schnarr,

Bernd; Ezernieks, Juris; Sebald, Walter; Duschl, Albert
CORPORATE SOURCE: Physiologische Chemie II, Theodor-Boveri-Inst. Biowissenschaften (Biozentrum), Wurzburg, D-97074, Germany
SOURCE: J. Biol. Chem. (1997), 272(3), 1480-1483
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We characterize here a highly efficient antagonist for interleukin-4 (IL-4) in the mouse system. In this double mutant of the murine IL-4 protein, both glutamine 116 and tyrosine 119 were substituted by aspartic acid residues. This variant (QY) bound with similar affinity to the IL-4 receptor .**alpha**. subunit as wild type IL-4 without inducing cellular responses. In contrast, QY completely inhibited in a dose-dependent manner the IL-4-induced proliferation of lipopolysaccharide-stimulated murine splenic B-cells, of the murine T cell line CTLL-2, and of the murine pre-B-cell line BA/F3. QY also inhibited the IL-4-stimulated up-regulation of CD23 expression by lipopolysaccharide-stimulated murine splenic B-cells and abolished tyrosine phosphorylation of the transcription factor Stat6 and the tyrosine kinase Jak3 in IL-4-stimulated BA/F3 cells. Selective inhibition of IL-4 may be beneficial in T-helper cell type 2-dominated diseases, like type I hypersensitivity reactions or helminthic infections. The QY mutant could be an attractive tool to study in vivo the therapeutic potential of IL-4 antagonists in mouse systems.

L131 ANSWER 14 OF 50 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 13
ACCESSION NUMBER: 1994:577324 CAPLUS
DOCUMENT NUMBER: 121:177324
TITLE: Site-Specific Conjugation to **Interleukin 4** Containing **Mutated** Cysteine Residues Produces **Interleukin 4** -Toxin Conjugates with Improved Binding and Activity
AUTHOR(S): Kreitman, Robert J.; Puri, Raj K.; Leland, Pamela; Lee, Byungkook; Pastan, Ira
CORPORATE SOURCE: Division of Cancer Biology, National Cancer Institute, Bethesda, MD, 20892, USA
SOURCE: Biochemistry (1994), 33(38), 11637-44
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Fusion of a ligand to another protein frequently impairs the binding of the ligand. Recombinant toxins composed of mutants of Pseudomonas exotoxin (PE) fused to the C-terminus of human interleukin 4 (IL4) are cytotoxic to IL4 receptor- (IL4R-) bearing tumor cells but bind to the IL4R with only 1% the affinity of IL4. The authors have developed a method to connect a toxin to a ligand which allows the junction to be moved to a location on the ligand which would minimize the binding impairment. The authors designed mutants of IL4 in which residue 28, 38, 68, 70, 97, or 105 was substituted with cysteine. All purified mutants bound to the IL4R with 60-100% the affinity of IL4, indicating that the IL4 structure was essentially unchanged. The IL4 mutants were then each conjugated through a disulfide bond to PE35, a truncated form of PE which contains a single cysteine. IL4 conjugated to PE35 at residue 28, 38, or 105 of IL4 bound with 10-fold improved affinity and was 10-fold more cytotoxic than the recombinant IL4-toxin in which PE is fused to position 129 at the C-terminus of IL4. IL4 contg. PE35 conjugated at position 68, 70, or 97 had lower binding affinity and cytotoxic activity. These

results indicate that the location of the ligand-protein junction can be selectively moved to enhance conjugate effectiveness, and implications could be made regarding which regions of IL4 are important for binding.

L131 ANSWER 15 OF 50 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 14
ACCESSION NUMBER: 1994:105024 CAPLUS
DOCUMENT NUMBER: 120:105024
TITLE: Mutant cytokines having increased receptor affinity
INVENTOR(S): Lakkis, Fadi; Murphy, John R.
PATENT ASSIGNEE(S): University Hospital, USA
SOURCE: PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9321308	A1	19931028	WO 1993-US3613	19930416
W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9342891	A1	19931118	AU 1993-42891	19930416
PRIORITY APPLN. INFO.:			US 1992-870500	19920417
			WO 1993-US3613	19930416

AB A variant of a naturally-occurring cytokine has a neutral amino acid substituted for a neg.-charged amino acid within 2 amino acids immediately upstream or downstream from a Phe-Leu or Tyr-Leu sequence in a helical domain. The variant cytokine has an increased affinity for the receptor. A hybrid mol. comprises a receptor-binding portion of the variant cytokine joined together covalently with a mol. having enzymic activity (e.g., a cytotoxin). The hybrid mol. decreases cell viability. DAB389-mIL-4, a fusion protein contg. diphtheria toxin having a deletion of 97 amino acids (Thr387-His485; the generalized cell binding domain) replaced with murine IL-4, was altered by site-directed and in-frame deletion mutagenesis to alter the mIL-4 portion of DAB389-mIL-4. Deletion of the C-terminal 15 amino acids of mIL-4; substitution of Phe496 with Pro, Ala, or Tyr; or substitution of Leu497 with Ala or Glu decreased binding to the mIL-4 receptor and cytotoxicity. In contrast, the substitution of the neg.-charged residue Asp495 with Asn resulted in a 4-fold increase in cytotoxic potency and binding affinity to mIL-4 receptor bearing cells in vitro.

L131 ANSWER 16 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:286088 CAPLUS
DOCUMENT NUMBER: 130:295558
TITLE: **Interleukin 4** derivatives showing low-**affinity** and short-term interaction with the common .gamma. chain of the **interleukin** receptors
INVENTOR(S): Sebald, Walter
PATENT ASSIGNEE(S): Bayer Aktiengesellschaft, Germany
SOURCE: PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9920765	A1	19990429	WO 1998-EP6448	19981012
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 911401	A1	19990428	EP 1997-118219	19971021
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9897495	A1	19990510	AU 1998-97495	19981012
EP 1023446	A1	20000802	EP 1998-951511	19981012
R: DE, ES, FR, GB, IT				

PRIORITY APPLN. INFO.: EP 1997-118219 A 19971021
WO 1998-EP6448 W 19981012

AB Human IL-4 (IL-4), one of the small 4-helix-bundle cytokines, uses the specific IL-4 receptor chain together with the common .gamma. chain (.gamma.c) for transmembrane signaling. The ligand-binding properties of .gamma.c, which are presently poorly understood, were analyzed by biosensor techniques employing recombinant ectodomains gamex (.gamma.c) and IL4-BP (a) of the receptor chains. The formation and decay of a ternary complex between solute gamex and IL-4 liganded IL4-BP could be established to exhibit a low **affinity** (Kd = 3.mu.M) as well as a short half life t1/2 = 7s. This binding **affinity** resulted largely from the interaction of gamex with IL-4 and not from a direct contact of IL4-BP and gamex, since the binary complex between solute gamex and immobilized IL-4 showed an only 50-fold greater Kd of 150 .mu.M. The IL-4 residues involved in gamex binding were identified by means of an alanine-scanning mutational approach. A functional gamex binding IL-4 epitope in which residues isoleucine-11, asparagine-15, and tyrosine-124 play significant roles is proposed. Even IL-4 variants which bind gamex 300-fold weaker than IL-4 with a dissocn. half life t1/2 of less than 1s, retained a substantial T-cell proliferative activity. These findings suggest that low **affinity** .gamma.c binding and short half lives of the heterodimeric a/.gamma.c receptor complex are sufficient for initiating IL-4 dependent signal transduction.

REFERENCE COUNT: 8
REFERENCE(S):
(1) Bayer Corporation; WO 9803654 A 1998 CAPLUS
(3) Duschl, A; EUROPEAN JOURNAL OF CYTOKINE NETWORK 1996, V7(1), P37 CAPLUS
(4) Kruse, N; EMBO JOURNAL 1993, V12(13), P5121 CAPLUS
(5) Letzelter, F; EUROPEAN JOURNAL OF BIOCHEMISTRY 1998, V257(1), P11 CAPLUS
(7) Ramanathan, L; BIOCHEMISTRY 1993, V32, P3549 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L131 ANSWER 17 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:772199 CAPLUS
DOCUMENT NUMBER: 132:19601
TITLE: Method of determining the risk of developing atopic allergy based on gene sequence
INVENTOR(S): Izuhara, Kenji; Hamazaki, Naotaka
PATENT ASSIGNEE(S): Daiichi Seiyaku Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 11 pp.
CODEN: JKXXAF

Searched by Barb O'Bryen, STIC 308-4291

DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 11332567	A2	19991207	JP 1998-140703	19980522

AB A method of detg. the risk of developing atopic allergy in patients is reported. The method relies on the correlation between Valine 50 to Isoleucine (V50I) mutation in interleukin-4 (IL-4) receptor **.alpha** .-chain and the pathogenesis of atopic asthma. The presence of V50I mutation in IL-4 receptor **.alpha**.-chain in the sample collected from the patient is examd. PCR based genetic method as reported is preferred, but alternative methods such as using monoclonal antibody are also possible. Various applications of the discovery such as use of IL-4 receptor ligand antagonists as therapeutic or preventive agent for atopic allergy are claimed. Atopic dermatitis, atopic asthma, and Hay fever are the particular afflictions covered.

L131 ANSWER 18 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:492824 CAPLUS
DOCUMENT NUMBER: 129:213163
TITLE: Mutational analysis of the STAT6 SH2 domain
AUTHOR(S): Mikita, Thomas; Daniel, Carla; Wu, Pengguang; Schindler, Ulrike
CORPORATE SOURCE: Tularik Inc., South San Francisco, CA, 94080, USA
SOURCE: J. Biol. Chem. (1998), 273(28), 17634-17642
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The SH2 domain of the STAT family of transcription factors is essential for STAT binding to phosphorylated cytoplasmic domains of activated cytokine receptors. Furthermore, the same domain mediates dimerization of activated STAT monomers, a prerequisite for DNA binding by this family of proteins. To identify amino acid residues within the STAT protein that mediate these various interactions, we have carried out an extensive mutational anal. of the Stat6 SH2 domain. Recombinant proteins carrying C-terminal deletions or double alanine substitutions were expressed in mammalian and insect cells and assayed for DNA binding, transcription activation, tyrosine phosphorylation, and the ability to interact with a tyrosine-phosphorylated peptide derived from the interleukin-4 receptor signaling chain. From these studies, we have identified amino acids that are required for both DNA binding and interleukin-4 receptor interaction, as well as residues that when mutated impair only one of the two functions. Our results suggest that the structural homol. between the SH2 domain of Stat6 and that of the distantly related Src protein may be higher than predicted on the basis of primary amino acid sequence comparisons. However, the two types of SH2 domains may differ at their C-terminal ends.

L131 ANSWER 19 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:534611 CAPLUS
DOCUMENT NUMBER: 129:243909
TITLE: An immune cell-selective interleukin 4 **agonist**
AUTHOR(S): Shanafelt, Armen B.; Forte, Carla P.; Kasper, James J.; Sanchez-Pescador, Lisa; Wetzel, Monte; Gundel, Robert; Greve, Jeffrey M.
CORPORATE SOURCE: Bayer Corporation, Pharmaceutical Division,

Searched by Barb O'Bryen, STIC 308-4291

Biotechnology, Berkeley, CA, 94710, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1998), 95(16), 9454-9458
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Interleukin 4 (IL-4) is a pleiotropic cytokine. Of the cell types responsive to IL-4, T cells express one IL-4 receptor (IL-4R) type, IL-4R.**alpha**./IL-2R.**gamma**. (class I IL-4R), whereas endothelial cells express another type, IL-4R.**alpha**./IL-13R.**alpha**. (class II IL-4R). It was hypothesized that IL-4 variants could be generated that would be selective for cell types expressing the different IL-4Rs. A series of IL-4 muteins were generated that were substituted in the region of IL-4 implicated in interactions with IL-2R.**gamma**.. These muteins were evaluated in T cell and endothelial cell assays. One of these muteins, contg. the mutation Arg-121 to Glu (IL-4/R121E), exhibited complete biol. selectivity for T cells, B cells, and monocytes, but showed no activity on endothelial cells. Receptor binding studies indicated that IL-4/R121E retained phys. interaction with IL-2R.**gamma**. but not IL-13R.**alpha**.; consistent with this observation, IL-4/R121E was an antagonist of IL-4-induced activity on endothelial cells. IL-4/R121E exhibits a spectrum of activities in vitro that suggest utility in the treatment of certain autoimmune diseases.

L131 ANSWER 20 OF 50 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:264057 CAPLUS
DOCUMENT NUMBER: 129:15221

TITLE: An antagonistic **IL-4** **mutant** prevents type I allergy in the mouse: inhibition of the **IL-4/IL** -13 **receptor** system completely abrogates humoral immune response to allergen and development of allergic symptoms in vivo

AUTHOR(S): Grunewald, Susanne M.; Werthmann, Antje; Schnarr, Bernd; Klein, C. Eberhard; Brocker, Eva B.; Mohrs, Markus; Brombacher, Frank; Sebald, Walter; Duschl, Albert
CORPORATE SOURCE: Biozentrum, Physiologische Chemie II, Universitat Wurzburg, Wurzburg, D-97074, Germany

SOURCE: J. Immunol. (1998), 160(8), 4004-4009
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have analyzed in vivo effects of the murine IL-4 mutant Q116D/Y119D (QY), which forms unproductive complexes with IL-4R.**alpha**. and is an antagonist for IL-4 and IL-13 in vitro. Treatment of BALB/c mice with QY during immunization with OVA completely inhibited synthesis of OVA-specific IgE and IgG1. BALB/c-derived knockout mice lacking either IL-4 or IL-4R.**alpha**. also did not develop specific IgE or IgG1, but mounted a much stronger IgG2a and IgG2b response than wild-type mice. In contrast, QY treatment of normal BALB/c mice suppressed specific IgG2a, IgG2b, and IgG3 synthesis, which may indicate the development of tolerance toward the allergen. Assocd. with the lack of IgE synthesis in QY-treated wild-type mice and in IL-4-/- mice used as a control was the failure to develop immediate cutaneous hypersensitivity or anaphylactic shock upon rechallenge. Interestingly, QY treatment also inhibited humoral immune responses and allergic reactivity in SJL/J mice, a strain that did not produce IgE, but displayed IgE-independent mast cell degranulation mediated by specific IgG1. We conclude that QY inhibits Ag-specific

humoral immune responses and allergic symptoms mediated either by IgE or IgG1. It needs to be clarified how QY abrogates synthesis of IgG2a, IgG2b, and IgG3, but the induction of tolerance toward nonhazardous protein Ags should be advantageous for therapy of atopic disorders and other Th2-dominated diseases.

L131 ANSWER 21 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:651605 CAPLUS
DOCUMENT NUMBER: 130:23950
TITLE: Specific antagonism of type I **IL-4** **receptor** with a **mutated** form of murine **IL-4**
AUTHOR(S): Schnare, Markus; Blum, Horst; Juttner, Stefan; Rollinghoff, Martin; Gessner, Andre
CORPORATE SOURCE: Institut fur Klinische Mikrobiologie, Immunologie, und Hygiene, Universitat Erlangen-Nurnberg, Erlangen, 91054, Germany
SOURCE: J. Immunol. (1998), 161(7), 3484-3492
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB IL-4 is a pleiotropic cytokine that is essential for the differential of Th2 cells and is critically involved in the pathogenesis of certain infectious and allergic diseases. The authors have produced and functionally characterized a mutant of murine IL-4 (IL-4.Y119D) as a potential antagonist of IL-4. The anal. of IL-4R binding revealed no differences between wild-type and mutated IL-4. Despite this finding, IL-4.Y119D was unable to induce proliferation of several IL-4-responsive T cell lines mediated via the type I IL-4R (IL-4R.**alpha**./common . **gamma**. chain (.**gamma**.c chain)) and specifically inhibited the proliferative effect of wild-type IL-4. In contrast, with IL-4.Y119D the authors found induction of MHC class II and CD23 mols. on resting splenic B cells as well as proliferation of B9 plasmacytoma cells. In addn., IL-4.Y119D induced mRNA for sol. IL-4R, leading to the release of sol. IL-4R protein by spleen cells. In macrophages, mutated IL-4 in combination with IFN-.**gamma**. induced TNF-.**alpha** .-dependent killing of Leishmania major parasites such as wild-type IL-4. The agonistic effects of IL-4.Y119D were obsd. on cells expressing the IL-13R .**alpha**.-chain, including an IL-13R .**alpha** .-chain transfected T cell line, but were absent in T cells that lack this mol., indicating that IL-4.Y119D conveys its activity via the type II IL-4R (IL-4.**alpha**./IL-13R.**alpha**.). The described IL-4 mutant, therefore, represents a new tool to use in dissecting different IL-4 functions that are mediated by either type I or type II IL-4R complexes.
REFERENCE COUNT: 49
REFERENCE(S): (1) Aarden, L; Eur J Immunol 1987, V17, P1411 CAPLUS
(4) Aversa, G; J Exp Med 1993, V178, P2213 CAPLUS
(5) Blum, H; J Immunol 1996, V157, P1846 CAPLUS
(6) Bogdan, C; Ann NY Acad Sci 1993, V685, P713 CAPLUS
(7) Bogdan, C; Curr Opin Immunol 1996, V8, P517 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L131 ANSWER 22 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:740114 CAPLUS
DOCUMENT NUMBER: 130:94063
TITLE: Cytokine **antagonists** and allergy
AUTHOR(S): Grunewald, Susanne M.; Brocker, Eva B.; Sebald, Walter; Duschl, Albert
CORPORATE SOURCE: Department of Dermatology, University of Wurzburg,

Wurzburg, 97080, Germany
SOURCE: Eur. Cytokine Network (1998), 9(Suppl. 3), 92-94
CODEN: ECYNEJ; ISSN: 1148-5493
PUBLISHER: John Libbey Eurotext
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review and discussion with 16 refs. Cytokines esp. involved in development and maintenance of allergic reactions are interleukin 4, IL-5 and eotaxin. Here, an antagonistic variant of mouse IL-4 was produced, QY, which binds to IL-4R.**alpha**. but has no detectable biol. activity. The results show that treatment of mice with QY during the sensitization phase completely prevented the development of ovalbumin-specific IgE and IgG1 and led to failure to develop immediate cutaneous hypersensitivity or anaphylactic shock upon rechallenge.
REFERENCE COUNT: 16
REFERENCE(S): (1) Burstein, H; J Immunol 1991, V147, P2950 CAPLUS
(2) Foster, P; J Exp Med 1996, V183, P195 CAPLUS
(3) Gonzalo, J; J Clin Invest 1996, V98, P2332 CAPLUS
(4) Grunewald, S; J Biol Chem 1997, V272, P1480 CAPLUS
(6) Kopf, M; Immunity 1996, V4, P15 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L131 ANSWER 23 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:226814 CAPLUS
DOCUMENT NUMBER: 124:258206
TITLE: **Interleukin**-13 (**IL**-13) induces **IL**-1 **receptor antagonist** gene expression and protein synthesis in peripheral blood mononuclear cells: inhibition by an **IL -4 mutant** protein
AUTHOR(S): Vannier, Edouard; de Waal Malefyt, Rene; Salazar-Montes, Adriana; de Vries, Jan E.; Dinarello, Charles A.
CORPORATE SOURCE: Dep. Med., Tufts Univ. School Med. New England Med. Center, Boston, MA, USA
SOURCE: Blood (1996), 87(8), 3307-15
CODEN: BLOOAW; ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Interleukin-13 (IL-13) belongs to the IL-4 gene family. Like IL-4, IL-13 induces IL-1 receptor antagonist (IL-1Ra) synthesis with no effect on IL-1.beta. synthesis. We investigated whether IL-13 induces IL-1Ra synthesis via a pathway similar to IL-4. In human peripheral blood mononuclear cells, IL-13 (1 to 100 ng/mL) alone induced IL-1Ra synthesis in a dose-dependent manner. A single amino acid mutant form of IL-4 (hIL-4.Y124D) induced IL-1Ra synthesis, acting as a partial agonist. However, hIL-4.Y124D inhibited IL-1Ra synthesis induced by either IL-4 or IL-13. IL-13 alone induced accumulation of IL-1Ra mRNA. Furthermore, IL-13 reduced steady-state levels for IL-1.beta. mRNA but enhanced those for IL-1Ra mRNA in cells stimulated with lipopolysaccharide (LPS) or IL-1. **alpha**.. Accordingly, IL-13 suppressed IL-1.beta. synthesis but enhanced IL-1Ra synthesis in these cells. IL-13 reduced the stability of IL-1.beta. mRNA (2.9 v 1.7 h) but failed to modify the stability of IL-1Ra mRNA (2.7 v 2.5 h). Moreover, IL-13 induced transcriptional activation of the IL-1Ra gene, but reduced IL-1.beta. gene transcriptional activation of the IL-1Ra gene, but reduced IL-1B gene transcription. Our results suggest that the commonality between IL-13 and IL-4 in inducing IL-1Ra synthesis results from the engagement of a subunit common to both receptors.

L131 ANSWER 24 OF 50 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:464053 CAPLUS
DOCUMENT NUMBER: 125:112620
TITLE: Monocyte function in a severe combined immunodeficient patient with a donor splice site mutation in the Jak3 gene
AUTHOR(S): Villa, Anna; Sironi, Marina; Matteucci, Cristian; Notarangelo, Luigi D.; Vezzoni, Paolo; Mantovani, Alberto
CORPORATE SOURCE: Instituto di Richerche Farmacologiche M. Negri, Milan, 20157, Italy
SOURCE: Blood (1996), 88(3), 817-823
CODEN: BLOOAW; ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Janus kinase-3 (Jak3) is a nonreceptor tyrosine kinase functionally coupled to cytokine receptors which share a "common" .**gamma**. chain (.**gamma**.c). Mutations in .**gamma**.c and Jak3 genes have been identified in X-linked and autosomal severe combined immune deficiency (SCID), resp. Jak3 is expressed and activated in myelomonocytic cells. The present study was designed to define the structural alteration responsible for lack of Jak3 in a patient with autosomal SCID and to characterize monocyte function in the absence of this signal transduction element, as well as to establish the whole exon-intron structure. Polymerase chain reaction anal., performed with primers designed on exon sequences, identified 20 exons spanning approx. 15 kb. These primers, or others designed on the flanking sequences provided in the present report, can be used to amplify the whole gene, allowing the definition of the mol. defects in all cases, including parental diagnosis, in which transcript anal. is not possible. On this basis, the deletion transcript found at the homozygous state in patient CM, with both his consanguineous parents being heterozygous for the deletion, was assocd. with mutation (T to C) of a splice donor site of intron 16 that was also detected in his mother's DNA. Monocytes from Jak3-SCID showed normal cytokine prodn. in response to interleukin-4 (IL-4) (release of IL-1 receptor antagonist) and IL-2 (release of tumor necrosis factor-.**alpha**. and IL-8). Lipopolysaccharide-induced cytokine prodn. was also normal and was blocked by IL-4 in Jak3-SCID monocytes. Interferon-.**gamma**. induced augmented expression of major histocompatibility class II in Jak3-SCID monocytes. These data indicate that Jak3, expressed and activated in myelomonocytic cells, is dispensable for monocyte differentiation and responsiveness to cytokines that interact with .**gamma**.c receptors as well as to other regulatory signals.

L131 ANSWER 25 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:863163 CAPLUS
DOCUMENT NUMBER: 123:254242
TITLE: High activity suppression of myeloid progenitor proliferation by chimeric **mutants** of **interleukin** 8 and platelet factor **4**
AUTHOR(S): Daly, Thomas J.; LaRosa, Gregory J.; Dolich, Sylvia; Maione, Theodore E.; Cooper, Scott; Broxmeyer, Hal E.
CORPORATE SOURCE: Repligen Corp., Cambridge, MA, 02139, USA
SOURCE: J. Biol. Chem. (1995), 270(40), 23282-92
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The proliferation of human myeloid progenitor cells is neg. regulated in the presence of certain members of the chemokine family of mols. This includes interleukin 8 (IL-8) and platelet factor 4 (PF4), which in combination are able to synergize, resulting in cell suppression at very

low concns. of these mols. A series of PF4 and IL-8 mutant proteins were analyzed in an in vitro colony formation assay for myeloid progenitor cells to assess domains of these proteins that are required for activity. Mutation of either of the two DLQ motifs within PF4 resulted in an inactive protein; perturbations within the IL-8 dimer interface region also resulted in mutants that were incapable of suppressing colony formation. A class of chimeric mutants consisting of domains of either PF4 and IL-8, Gro-.alpha. and PF4, or Gro-.beta. and PF4 were obsd. to inhibit myeloid cell proliferation at concns. which were between 500- and 5000-fold lower than either the IL-8 or PF4 wild-type proteins alone. These chimeric mutants possessed activities that were comparable to or better than the activity obsd. when IL-8 and PF4 were added together in vitro. One of these highly active chimeric proteins was obsd. to be 1000-fold more active than either IL-8 or PF4 alone in suppressing not only the proliferation but also the cell cycling of myeloid progenitor cells following i.v. injection of the mutant into mice. Examn. of addnl. IL-8-based mutants in the colony formation assay, which centered on the perturbation of the amino-terminal "ELR" motif, resulted in the observation that the highly active IL-8 mutant required both aspartic acid at amino acid residue 4 and either glutamine or asparagine at residue 6. Single mutations at either of these positions resulted in mutants with myelosuppressive activity equiv. to wild-type IL-8. Mutants such as IL-8M1 and IL-8M10 were obsd. to be significantly reduced in their ability to activate isolated human neutrophils, suggesting that sep. mechanisms may exist by which myeloid progenitor cells and neutrophils are affected by chemokines.

L131 ANSWER 26 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:418069 CAPLUS
DOCUMENT NUMBER: 122:185133
TITLE: An antagonistic **mutant** of **interleukin-4** fails to recruit .gamma.c into the receptor complex. Characterization by specific crosslinking
AUTHOR(S): Duschl, Albert
CORPORATE SOURCE: Physiol. Chem. II, Theodor-Boveri-Inst. Biowissenchaften, Wuerzburg, Germany
SOURCE: Eur. J. Biochem. (1995), 228(2), 305-10
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The receptor for interleukin-4 (IL-4) appears to be a heterodimer of the IL-4-binding protein IL-4R.alpha. and the .gamma.c chain. Mutations of IL-4 have previously identified a region of IL-4 essential for signaling, which was suggested to bind .gamma.c. Here it is shown by crosslinking of radiolabeled ligand and pptn. with specific antibodies that mutations in the IL-4 signaling site prevent assocn. of .gamma.c, but not binding to IL-4R.alpha.. This demonstrates that an intact signaling site of IL-4 is required to recruit .gamma.c into the receptor complex, while specific mutants are antagonists because they fail to achieve this.

L131 ANSWER 27 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:215040 CAPLUS
DOCUMENT NUMBER: 120:215040
TITLE: **Mutational** analysis of a critical signaling domain of the human **interleukin 4** receptor
AUTHOR(S): Seldin, David C.; Leder, Philip
CORPORATE SOURCE: Howard Hughes Med. Inst., Harvard Med. Sch., Boston, MA, 02115, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1994), 91(6), 2140-4

Searched by Barb O'Bryen, STIC 308-4291

CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The human interleukin 4 receptor (hIL-4R) is a member of a superfamily of cytokine receptors defined by conserved features of their extracellular domains. The intracellular domains of the hIL-4R and of other members of this family lack any recognizable enzymic motifs, though ligand-dependent tyrosine phosphorylation of these receptors has been obsd. Recent studies have suggested that serine-rich and acidic domains within the cytoplasmic portions of cytokine receptors might be required for signal transduction. Using deletion and truncation mutants of the hIL-4R, the authors have explored an essential 39-amino acid signaling domain that is rich in acidic amino acid residues and in serine residues that form consensus phosphorylation sites for known serine/threonine kinases. To assess the contribution of these motifs to signaling, the authors engineered site-directed mutants of these residues. Surprisingly, cells expressing mutant hIL-4R lacking either the serine or the acidic amino acids retain the ability of cells expressing the wild-type receptor to proliferate in hIL-4. Furthermore, receptors in which all six cytoplasmic tyrosines are absent can function, suggesting that tyrosine phosphorylation of the receptor may be an epiphenomenon rather than a requisite event in signaling.

L131 ANSWER 28 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:515340 CAPLUS
DOCUMENT NUMBER: 119:115340
TITLE: Region of cytoplasmic domain of the human interleukin-4 receptor (IL-4R), as antagonists of IL-4
INVENTOR(S): Harada, Nobuyuki; Izuhara, Kenji; Miyajima, Atsushi; Howard, Maureen C.
PATENT ASSIGNEE(S): Schering Corp., USA
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9311234	A1	19930610	WO 1992-US9897	19921124
W: AU, BB, BG, BR, CA, CS, FI, HU, JP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD, UA, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
AU 9331396	A1	19930628	AU 1993-31396	19921124
EP 615546	A1	19940921	EP 1992-925279	19921124
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07505047	T2	19950608	JP 1992-510142	19921124

PRIORITY APPLN. INFO.: US 1991-803621 19911127
WO 1992-US9897 19921124

AB Antagonists of human IL-4 are provided which are based upon a crit. region of the cytoplasmic domain of the human IL-4R. Also provided are compns. and methods for inhibiting the biol. activity of human IL-4. Plasmids were constructed contg. the human IL-4R cDNA and deletion mutants along with the neo resistant gene. Plasmid DNAs were transfected into murine pro-B Ba/F3 cells by electroporation and stable transfectants were assayed for I-4R activity. A short segment encoding 41 amino acid residues was identified which is crit. for signal transduction. Results also demonstrate that high affinity binding to IL-4 can still be obsd. on stable transfectants expressing a mutant IL-4R cDNA which is not capable

of growth signal transduction, and that such cells are capable of internalizing IL-4.

L131 ANSWER 29 OF 50 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1992:569241 CAPLUS
DOCUMENT NUMBER: 117:169241
TITLE: Conversion of human interleukin-4 into a high affinity antagonist by a single amino acid replacement
AUTHOR(S): Kruse, N.; Tony, H. P.; Sebald, W.
CORPORATE SOURCE: Theodor-Boveri-Inst. Biowiss., Univ. Wuerzburg, Wuerzburg, D-8700, Germany
SOURCE: EMBO J. (1992), 11(9), 3237-44
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Interleukin-4 (IL-4) represents a prototypic lymphokine. It promotes differentiation of B-cells and the proliferation of T- and B-cells, and other cell types of the lymphoid system. An antagonist of human IL-4 was discovered during the studies presented here after Tyr124 of the recombinant protein had been substituted by an aspartic acid residue. This IL-4 variant, Y124D, bound with high affinity to the IL-4 receptor (KD = 310 pM), but retained no detectable proliferative activity for T-cells and inhibited IL-4-dependent T-cell proliferation competitively (Ki = 620 pM). The loss of efficacy in variant Y124D was estd. to be >100-fold on the basis of a weak partial agonist activity for the very sensitive induction of CD23 pos. B-cells. The substitution of Tyr124 by either phenylalanine, histidine, asparagine, lysine or glycine resulted in partial agonist variants with unaltered receptor binding affinity and relatively small deficiencies in efficacy. Thus, high affinity binding and signal generation can be uncoupled efficiently in a ligand of a receptor belonging to the recently identified hematopoietin receptor family. In addn. it is shown for the first time, that a powerful antagonist acting on the IL-4 receptor system can be derived from the IL-4 protein.

L131 ANSWER 30 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 6
ACCESSION NUMBER: 1998:475767 BIOSIS
DOCUMENT NUMBER: PREV199800475767
TITLE: The interleukin-4 site-2 epitope determining binding of the common receptor **gamma** chain.
AUTHOR(S): Letzelter, Felix; Wang, Yonghong; Sebald, Walter (1)
CORPORATE SOURCE: (1) Theodor-Boveri-Institut Biowissenschaften, Physiologische Chemie II, Universitaet Wuerzburg, Am Hubland, D-97074 Wuerzberg Germany
SOURCE: European Journal of Biochemistry, (Oct. 1, 1998) Vol. 257, No. 1, pp. 11-20.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Human IL-4 (IL-4), one of the small four-helix-bundle cytokines, uses the specific IL-4 **receptor** a chain together with a promiscuous subunit, the common **gamma** chain (gammac) for transmembrane signaling. The ligand-binding properties of gammac, which are presently poorly understood, were analysed by biosensor techniques employing recombinant ectodomains of gammac and **alpha receptor** chains (HA-BP). The formation of a ternary complex between solute gammac ectodomain and IL-4 saturated IL-4-BP could be established to exhibit a high dissociation constant Kd = 3 muM and a short half life tau1/2 = 7 s. This binding **affinity** resulted to the major part from the interaction of gammac ectodomain with IL-4 and not from a direct contact of the ectodomains, since binding between solute gammac ectodomain and

IL-4 could be established (Kd about 150 muM), whereas no binding was found between the gammac ectodomain and HA-BP in the absence of IL-4. The IL-4 epitope involved in gammac ectodomain interaction (site 2) was identified by means of an alanine-scanning **mutational** approach. The **IL-4** site 2 comprised residues I11 and N15 on helix A together with Y124 on helix D as major binding determinants. The IL-4 alanine variants at site 2 generally showed response (EC.) was not altered by site-2 substitutions. The present results are in accordance with a two-step-dimerisation mechanism for IL-4 **receptor** activation, where solute IL-4 at physiological concentrations binds first via the high-**affinity** site 1 to the a chain only, since the **affinity** of IL-4 site 2 for gammac is too low. This site-2 **affinity** seems to be sufficient, however, to promote, in a second step, a productive association of gammac to an IL-4/**alpha** chain complex in the membrane.

L131 ANSWER 31 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 10
ACCESSION NUMBER: 1997:110985 BIOSIS
DOCUMENT NUMBER: PREV199799410188
TITLE: X-SCID B cells responses to interleukin-4 and interleukin-13 are mediated by a receptor complex that includes the interleukin-4 receptor **alpha** chain (p140) but not the **gamma**-c chain.
AUTHOR(S): Matthews, David J.; Hibbert, Linda; Friedrich, Karlheinz; Minty, Adrian; Callard, Robin E. (1)
CORPORATE SOURCE: (1) Immunobiol. Unit, Inst. Child Health, 30 Guilford St., London WC1N 1EH UK
SOURCE: European Journal of Immunology, (1997) Vol. 27, No. 1, pp. 116-121.
ISSN: 0014-2980.
DOCUMENT TYPE: Article
LANGUAGE: English
AB This study investigates the effect of interleukin (**IL**)-**4** **mutant** proteins and a monoclonal antibody to the IL-4 **receptor** a chain on IL-4 and IL-13 response by B cells from X-linked severe combined immunodeficiency (X-SCID) patients in which the common y chain (**gamma**-c chain) gene mutations have been fully characterized and no **gamma**-c chain expression was detected. In this **gamma**-c chain gene knockout model, it was confirmed that the **gamma**-c chain is essential for B cell responses to IL-2 but not for IL-4 or IL-13. Dose-response curves for X-SCID and normal B cell responses to IL-4 were indistinguishable, showing that the loss of the **gamma**-c chain did not diminish the sensitivity of B cells to **IL-4**. The **mutant** protein **IL-4**-Y124D and an antibody to the IL-4R **alpha** chain both inhibited responses of X-SCID B cells to IL-4 and IL-13, showing that X-SCID B cell responses to these cytokines are mediated by a **receptor** complex that includes the IL-4R **alpha** chain but not the **gamma**-c chain. Another **mutant** protein, **IL-4**-R88D, which has greatly reduced **affinity** for IL-4R-**alpha**, was found to inhibit responses by normal B cells to IL-4 but not to IL-13. IL-4-R88D did not, however, inhibit X-SCID B cell responses to IL-4. This result is consistent with IL-4-R88D inhibition of responses mediated by **receptor** complexes that include the **gamma**-c chain. We propose that X-SCID B cells responses to IL-4 are mediated by an IL-13 **receptor** complex comprised of the IL-4R **alpha** chain associated with the recently cloned IL-13R binding protein. This model has major implications for understanding normal B cell responses to IL-4.

L131 ANSWER 32 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 2000:88465 BIOSIS
DOCUMENT NUMBER: PREV200000088465
TITLE: Candidate genes and a genome-wide search in Italian families with atopic asthmatic children.
AUTHOR(S): Malerba, G.; Trabetti, E.; Patuzzo, C.; Lauciello, M. C.; Galavotti, R.; Pescollderungg, L.; Boner, A. L.; Pignatti, P. F. (1)
CORPORATE SOURCE: (1) Biology and Genetics, University of Verona, Strada Le Grazie 8, 37134, Verona Italy
SOURCE: Clinical and Experimental Allergy, (Dec., 1999) Vol. 29, No. Suppl. 4, pp. 27-30.
ISSN: 0954-7894.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB To identify genetic factors for susceptibility to atopy and asthma in childhood, 1083 subjects were identified, mainly from the Veneto region and Bolzano province in North-east Italy, of whom 817 were from 172 families with at least two affected people, 189 were sporadic cases, and 77 unrelated controls. All the subjects were characterized for clinical asthma (asthma), total serum IgE (IgE), skin prick test (SPT) reactivity to common aeroallergens and bronchial hyperresponsiveness (BHR) to methacoline test. Atopy was defined as SPT positivity and/or increased IgE levels. Several candidate genes were investigated, and genome-wide linkage analysis was been initiated. The high **affinity** IgE **receptor** beta chain (FcepsilonRIbeta) locus showed significant allele sharing in affected sib-pairs for BHR and for SPT positivity. Lymphotoxin **alpha** (Ltalpha) gene NcoI mutation showed a suggestive linkage with atopy, and the LTalphaNco1 2/2 genotype was found to be associated with increased total IgE levels in all females. No evidence for linkage or association of any phenotype to the tumour necrosis factor **alpha** (TNFalpha) - 308 **mutation** or to the **interleukin 4 receptor alpha** (IL-4Ralpha) Q576R **mutation** was found. BHR, asthma and increased IgE were found to be linked to X and Y long arm pseudoautosomal region (PAR2) markers. Initial data were also collected from linkage analysis with chromosome 12, 14, and 19, DNA markers. Non-parametric multipoint analysis provides preliminary evidence for linkage of asthma with D12S390, of atopy with D19S601, and of BHR with D14S617. These results suggest that several genetic factors contribute to different allergic asthma phenotypes in the population investigated.

L131 ANSWER 33 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:257635 BIOSIS
DOCUMENT NUMBER: PREV199800257635
TITLE: Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones.
AUTHOR(S): Fasler, Stephan; Aversa, Gregoria; De Vries, Jan E.; Yssel, Hans (1)
CORPORATE SOURCE: (1) INSERM U454, Hopital Arnaud de Villeneuve, 371 Ave. Doyen Gaston Giraud, 34295 Montpellier Cedex France
SOURCE: Journal of Allergy and Clinical Immunology, (April, 1998) Vol. 10, No. 4 PART 1, pp. 521-530.
ISSN: 0091-6749.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Background: Allergic disorders are characterized by IgE antibody responses to a multitude of allergens as a result of the ability of these antibodies to specifically bind to high-**affinity** IgE **receptors** on mast cells and basophils. This interaction results in **receptor**

activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate allergen-induced IgE responses. Objectives: Because of the central role of allergen-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have evaluated the possibility of inhibiting allergen-induced activation of these cells by using allergen-derived peptides that have been modified by single amino acid substitutions. Methods: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major allergen in house dust, were used in this study. Upon activation with Der p 1 or specific Der p 1-dervived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-**gamma** and IL-2, respectively, and furthermore give help to B cells for the production of IgE in vitro. Modified synthetic peptides were generated by the introduction of single amino acid substitutions into two different T-cell activation-inducing epitopes on Der p 1. The effects of these modified peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. Results: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-**gamma**, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-**gamma**, even at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to B cells for the production of IgE in vitro, even in the presence of exogenous **IL-4**. Conclusions: **Substitution** of certain **amino acid** residues in immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these modified peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by allergen-specific T-cell clones as well as on T cell-mediated IgE production by B cells. These findings suggest that modified peptides interfere with allergen-induced activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-B cell interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of allergen-specific TH2 cells.

L131 ANSWER 34 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:874 BIOSIS
DOCUMENT NUMBER: PREV199800000874
TITLE: Overexpression, purification, and use of a soluble human interleukin-4 receptor **alpha**-chain/Iggamma 1 fusion protein for ligand binding studies.
AUTHOR(S): Seipelt, Irene (1); Hoffmann, Silke H. (1); Schmidt, Juergen; Engels, Joachim W.; Beckers, Thomas
CORPORATE SOURCE: (1) Dep. Biochemistry, AWD, Dresden Germany
SOURCE: Biochemical and Biophysical Research Communications, (Oct. 20, 1997) Vol. 239, No. 2, pp. 534-542. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The pleiotropic cytokine IL-4 transmits cellular signals mainly via the IL-4 **receptor** complex, with the **alpha**-chain as the high **affinity** binding subunit. Here we describe the

overexpression of a soluble IL-4R **alpha**-chain (sIL-4R) as a fusion to immunoglobulin gamma1 heavy chain, consisting of the H-CH2-CH3 domains, in baby hamster kidney cells. The dimeric fusion protein named sIL-4R:Egamma1 was purified from culture supernatant by protein-A **affinity** chromatography, yielding up to 10 mg/l homogenous protein which was highly stable. The antibody-like features of the sIL-4R:Egamma1 fusion protein allowed immobilization on a biosensor matrix for surface plasmon resonance measurements by direct amine coupling as well as immobilization on microtiter plates coated with protein A for displacement binding. Kinetic parameters (kon and koff) for binding of **IL4** or the antagonistic **mutant** IL-4Y124D to the sIL-4R:Egamma1 fusion protein on the chip as determined with the BIAcore instrument showed a high **affinity** binding with KD = 239 +- 35 pM and KD=148 +- 33 pM, respectively. The extremely high kon rate and the relatively slow koff rate for both ligands highlighted the limits of the BIAcore technology. The binding **affinity** as calculated in displacement binding studies with biotinylated IL-4 was similar for IL-4 and IL-4Y124D (IC50=1.1nM), thus offering a simple alternative for initial characterization of **IL-4 mutants**.

L131 ANSWER 35 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1996:263169 BIOSIS
DOCUMENT NUMBER: PREV199698819298
TITLE: Interleukin-4 (IL-4) and IL-13 bind to a shared heterodimeric complex on endothelial cells mediating vascular cell adhesion molecule-1 induction in the absence of the common **gamma** chain.
AUTHOR(S): Schnyder, Bruno; Lugli, Serena; Feng, Ningping; Etter, Hansueli; Lutz, Ruedi A.; Ryffel, Bernhard; Sugamura, Kazuo; Wunderli-Allenspach, Heidi; Moser, Rene (1)
CORPORATE SOURCE: (1) Dep. Pharm., Biopharmacy, Federal Inst. Technol., Winterhurestr 190, CH-8057 Zurich Switzerland
SOURCE: Blood, (1996) Vol. 87, No. 10, pp. 4286-4295. ISSN: 0006-4971.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Interleukin-4 (IL-4) and IL-13 exert similar, nonadditive effects on endothelial cells, inducing vascular cell adhesion molecule-1 (VCAM-1) expression and subsequent transmigration of eosinophils. The **receptor** for IL-4 and IL-13 was described as a shared heteromultimeric complex in which the common **gamma**-chain (**gamma**-c) subunit was essential for activity. Endothelial cells bound both cytokines with high **affinity**; by flow cytofluorometry and reverse transcription-polymerase chain reaction (RT-PCR), they expressed IL-4 **receptor alpha** (IL4R-**alpha**) but did not express the **gamma**-c of the IL-2R. Radioligand cross-linking experiments followed by immunoprecipitation with the monoclonal antibody (MoAb) S697 to the IL-4R-**alpha** showed IL-4-specific binding at 130 kD, the IL-4R-**alpha**, and to a minor extent to a double band coimmunoprecipitated at 65 to 75 kD. (125I)IL-13 bound predominantly to the 65- to 75-kD band and with a trace amount of binding at 130 kD. However, no ligand-cross-linked **receptor** was precipitated by the MoAb S697, indicating a cognate novel IL-13-binding subunit. Excess unlabeled IL-4 completely displaced IL-13 binding. Similarly, nonsignaling **IL-4** (Y124D)-**mutant** abolished **IL-4**- and IL-13-mediated signal transduction. Unlabeled IL-13 competed successfully for IL-4 binding at 65 to 75 kD but was unable to completely displace IL-4 from its binding to the IL-4R-**alpha**. The MoAb TUGh4, specific for the **gamma** -c, failed to precipitate ligand-cross-linked IL-4R and IL-13R. Therefore, the subunit structure of the functional **receptors** for IL-4 and

IL-13 on human endothelial cells does not use or require the common **gamma**-c of the IL-2R.

L131 ANSWER 36 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1996:383099 BIOSIS
DOCUMENT NUMBER: PREV199699105455
TITLE: An improved circularly permuted interleukin 4-toxin is highly cytotoxic to human renal cell carcinoma cells.
AUTHOR(S): Puri, Raj K. (1); Leland, Pamela; Obiri, Nicholas I.; Husain, S. Rafat; Mule, Jim; Pastan, Ira; Kreitgman, Robert J.
CORPORATE SOURCE: (1) Lab. Molecular Tumor Biol., Div. Cellular Gene Therapies, Cent. Biologics Evaluation Res., Food Drug Adm., Natl. Inst. Health, Build. 29B, Room 2NN10, 29 Lincoln Drive MSC 4555, Bethesda, MD 20892 USA
SOURCE: Cellular Immunology, (1996) Vol. 171, No. 1, pp. 80-86. ISSN: 0008-8749.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We have previously demonstrated that a chimeric protein composed of human IL-4 and Pseudomonas exotoxin, termed IL4-PE-4E, is cytotoxic to primary cells derived from human renal cell carcinoma (RCC). To improve the cytotoxicity of IL4-toxins such as IL4-PE-4E and IL4-PE38KDEL to IL-4 **receptor** (IL-4R) positive tumor cells, a circularly permutated chimeric toxin was prepared by fusing a truncated PE gene encoding PE38KDEL 3' to a circularly permutated **IL-4** **mutant** gene encoding **IL4** amino acids 38-129, the linker GGNGG, and IL4 amino acids 1-37. The resulting chimeric protein, termed IL4(38-37)-PE38KDEL, was tested on five RCC cell lines and its cytotoxicity was compared to that of the native IL4-toxins IL4-PE-4E and IL4-PE38KDEL. IL4(38-37)-PE38KDEL was found to be 5 to 10 times more cytotoxic to all cell cultures tested compared to either native IL4-toxin. The cytotoxic activity of IL4(38-37)-PE38KDEL was competable by excess IL-4 and was confirmed by clonogenic assay. IL4(38-37)-PE38KDEL bound to IL-4R on RCC cells with 6- to 12-fold higher **affinity** than IL4-PE38KDEL or IL4-PE-4E. RCC tumor cells were found to lack the common **gamma** chain (**gamma**-c) of the IL-4R reported to be present on immune cells. The stable transfection of RCC cells with the **gamma**-c chain gene did not significantly change their sensitivity to IL4(38-37)-PE38YDEL. Taken together, our results indicate that the CPIL4-toxin IL4(38-37)-PE38KDEL is highly cytotoxic to human RCC cells due to increased binding **affinity** to IL4R while it is not cytotoxic or slightly cytotoxic to T and B cells, monocytic cell lines, and fresh resting or activated bone marrow-derived cells. The **gamma**-c does not seem to increase the internalization rate and/or processing of IL4-toxins in RCC cells. CPIL4-toxin may be a useful agent for the treatment of human RCC.

L131 ANSWER 37 OF 50 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1996:456265 BIOSIS
DOCUMENT NUMBER: PREV199699178621
TITLE: Modulation of the human IgE response.
AUTHOR(S): De Vries, J. E. (1); Yssel, H.
CORPORATE SOURCE: (1) DNAX Res. Inst., 901 California Avenue, Palo Alto, CA 94304 USA
SOURCE: European Respiratory Journal Supplement, (1996) Vol. 9, No. SUPPL. 22, pp. 58S-62S. ISSN: 0904-1850.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Studies on the immunological basis of allergic diseases have indicated

that enhanced production of the cytokines interleukin (IL)-4 and IL-13 and the reduced production of interferon-**gamma** (IFN-**gamma**) by allergen-specific T-cells contribute to enhanced immunoglobulin E (IgE) synthesis and the development of allergic disease in certain individuals. Therefore, inhibition of IL-4 and IL-13 synthesis or blocking of activities of these cytokines would be one approach to inhibiting IgE production. In the present communication, novel approaches toward this goal are discussed. It is shown that an **IL-4 mutant** protein, in which the tyrosine residue at position 124 is replaced by aspartic acid (IL-4.Y124D), binds with high **affinity** to the IL-4 **receptor**, without **receptor** activation. IL-4.Y124D acts as a potent antagonist both of IL-4 and IL-13 activity in vitro, and inhibits immunoglobulin G-4 (IgG-4) and IgE production induced by these cytokines. These data am compatible with the notion that the IL-4 and IL-13 **receptors** are complex **receptors**, which share a common component, which is required for signal transduction. In addition, it has been demonstrated that allergen-specific T-cells, belonging to the T-helper 2 (Th2) subset can be rendered anergic after incubation with allergen-derived peptides representing minimal T-cell activation inducing epitopes. These anergic Th2 cells failed to produce IL-4 and IL-13, and failed to proliferate after activation with allergen and antigen-presenting cells (APC). The anergized T cells also failed to give B-cells help in IgE synthesis, although they expressed normal levels of the CD40 ligand (CD40L). Exogenous IL-4 or IL-13 failed to restore IgE synthesis, indicating that in addition to CD40L other co-stimulatory signals are required for productive T-cell/B-cell interactions, resulting in IgE synthesis. IgE production was restored by exogenous IL-2, demonstrating that IL-2 reverses the nonresponsive state and helper function of these nonresponsive T-cells. It is tempting to speculate that induction of T-cell nonresponsiveness by allergen derived peptides may represent the underlying mechanisms for successful immunotherapy in allergenic patients.

L131 ANSWER 38 OF 50 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.DUPLICATE
ACCESSION NUMBER: 2001:32374206 BIOTECHNO
TITLE: A murine IL-4 receptor antagonist that inhibits IL-4- and IL-13-induced responses prevents antigen-induced airway eosinophilia and airway hyperresponsiveness
AUTHOR: Tomkinson A.; Duez C.; Cieslewicz G.; Pratt J.C.; Joetham A.; Shanafelt M.-C.; Gundel R.; Gelfand E.W.
CORPORATE SOURCE: Dr. E.W. Gelfand, 1400 Jackson Street, Denver, CO 80206, United States.
E-mail: gelfande@njc.org
SOURCE: Journal of Immunology, (01 MAY 2001), 166/9 (5792-5800), 68 reference(s)
CODEN: JOIMA3 ISSN: 0022-1767
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The closely related Th2 cytokines, IL-4 and IL-13, share many biological functions that are considered important in the development of allergic airway inflammation and airway hyperresponsiveness (AHR). The overlap of their functions results from the IL-4R **.alpha.**-chain forming an important functional signaling component of both the **IL-4** and IL-13 receptors. **Mutations** in the C terminus region of the **IL-4** protein produce **IL-4 mutants** that bind to the IL-4R **.alpha** .-chain with high **affinity**, but do not induce cellular responses. A murine **IL-4 mutant** (C118 deletion) protein (IL-4R antagonist) inhibited IL-4- and IL-13-induced

STAT6 phosphorylation as well as IL-4- and IL-13-induced IgE production in vitro. Administration of murine IL-4R antagonist during allergen (OVA) challenge inhibited the development of allergic airway eosinophilia and AHR in mice previously sensitized with OVA. The inhibitory effect on airway eosinophilia and AHR was associated with reduced levels of IL-4, IL-5, and IL-13 in the bronchoalveolar lavage fluid as well as reduced serum levels of OVA-IgE. These observations demonstrate the therapeutic potential of **IL-4 mutant** protein receptor antagonists that inhibit both IL-4 and IL-13 in the treatment of allergic asthma.

L131 ANSWER 39 OF 50 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28246983 BIOTECHNO
TITLE: The interleukin-4/interleukin-13 receptor of human synovial fibroblasts: Overexpression of the nonsignaling interleukin-13 receptor .alpha.
AUTHOR: Feng N.; Lugli S.M.; Schnyder B.; Gauchat J.-F.M.; Graber P.; Schlagenhauf E.; Schnarr B.; Wiederkehr-Adam M.; Duschl A.; Heim M.H.; Lutz R.A.; Moser R.
CORPORATE SOURCE: Dr. R. Moser, Inst. for Biopharmaceutical Research, P.O. Box 164, CH-9545 Waengi, Switzerland.
SOURCE: Laboratory Investigation, (1998), 78/5 (591-602), 58 reference(s)
CODEN: LAINAW ISSN: 0023-6837
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Interleukin (IL)-4 and IL-13 are known to bind to shared heteromultimeric receptor complexes of variable composition. Given the many regulatory effects of IL-4 and IL-13 on synovial cells, we aimed to characterize their IL-4/IL-13 receptor (R). Cultivated synovial fibroblasts expressed transcripts for IL-4R.alpha. and IL-13R.alpha.1, the human homolog of the recently cloned mouse IL-13R, but not the common .gamma.-chain of the IL-2R. In particular, IL-13R.alpha.2 mRNA, encoding a different IL-13R recently cloned from human renal carcinoma cells, was expressed at a strikingly high level. Correspondingly, a predominant protein migrating at 65 to 75 kd was cross- linked by iodinated IL-13 and was not cross-competed by an excess of unlabeled IL-4. However, by flow cytofluorometry, IL-13R.alpha.1 (detected by the anti-IL-13R.alpha.1 mAb 65) and IL-4R.alpha. (detected by the mAb S697) were expressed at similar low density. Radioligand binding studies revealed for both cytokines approximately 300 receptors/cell with similar high affinity. An additional class of IL-13Rs was identified after occupation of the shared high-affinity receptors by the nonsignaling, double-**mutant IL-4**.sup.1.sup.2.sup.1R.fwdarw.D, .sup.1.sup.2.sup.4Y.fwdarw.D (RY-IL-4). In these experiments, .sup.1.sup.2.sup.5I-IL-13 bound to a single receptor population with a K(d) of approximately 300 pM and approximately 5000 sites/cell, matching the published affinity of monomeric IL-13R.alpha.2 when expressed in COS7 cells. RY-IL-4 blocked the IL-4-and IL-13-mediated vascular cell adhesion molecule (VCAM)-1 expression and Stat6 activation, suggesting that the large number of high-affinity IL-13R.alpha.2 monomers are silent receptors, likely representing a decoy target for IL-13.

L131 ANSWER 40 OF 50 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-01645 BIOTECHDS
TITLE: New mutagenized interleukin 13 molecules for delivery of cytotoxins to cells over expressing IL13 **receptors**; **mutant** interleukin-13, toxin protein fusion

protein, used to deliver toxins to cancer cell overexpressing interleukin-13 **receptor**

AUTHOR: Debinski W
PATENT ASSIGNEE: Pennsylvania-State-Res.Found.
LOCATION: University Park, PA, USA.
PATENT INFO: WO 9951643 14 Oct 1999
APPLICATION INFO: WO 1999-US7188 31 Mar 1999
PRIORITY INFO: US 1998-54711 3 Apr 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-633731 [54]

AB A targeting ligand, particularly a mutated interleukin-13 (IL-13) molecules, containing at least one **mutation** in a domain that interacts with the human **interleukin-4** **receptor** subunit hIL4R-beta, is claimed. The ligand is a chimeric molecule with the formula R+1(L)-j-(R+2)-n, in which R+1 is the **mutant** IL-13, R+2 is an effector molecule, j and n are 0 or 1 and L is a linker. Also claimed is a specific binding moiety containing a **mutant** IL-13, a means of delivering an effector molecule to a cell containing an IL-13 **receptor**, using the ligand, and a means of inhibiting growth in a cell expressing IL-13 **receptor**, using a cytotoxic molecule attached to the **mutant** IL-13. The ligands have an increased **specificity** for cancer cells, and can be used in delivering effector molecules, particularly toxins, to cancer cells. The **mutation** in the IL-13 molecule is preferably conversion of amino acid 13 to a basic amino acid, arginine or lysine, or amino acid 66, 69, 109 or 112 to aspartic acid. The effector molecule is preferably a Diptheria toxin or Pseudomonas toxin that lacks the Ia domain. The fusion protein is produced by recombinant DNA technology. (57pp)

L131 ANSWER 41 OF 50 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-04805 BIOTECHDS
TITLE: New **interleukin-4** **mutein** with increased **affinity** for its **receptor**; used for immune disorder, cancer, allergy, allergic or inflammatory disease, e.g. asthma therapy
AUTHOR: Greve J; Shanafelt A B; Roczniak S
PATENT ASSIGNEE: Bayer
LOCATION: Pittsburgh, PA, USA.
PATENT INFO: WO 9803654 29 Jan 1998
APPLICATION INFO: WO 1997-US11909 9 Jul 1997
PRIORITY INFO: US 1996-687803 19 Jul 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-120778 [11]

AB A new human recombinant **interleukin-4** (**IL-4**) **mutein** has a defined 153 amino acid protein sequence with an **amino acid substitution** in the binding surface of either the A- or C-alpha helices of wild-type **IL-4**, where the **mutein** binds to the **IL-4** **receptor** with greater **affinity** than wild-type **IL-4**. The **mutein** may have substitutions R121D and Y124D in the D-helix of wild-type **IL-4**. Also claimed are a 462 bp DNA sequence encoding the **IL-4** **mutein** and a host cell transformed with the DNA. A new method for determining the ability of the **mutein** to bind the **receptor** involves: introducing into a FlashPlate coated with streptavidin, a **receptor** chain binding portion (RCBP) with a peptide tag able to bind to streptavidin, a radiolabeled native ligand with **affinity** for the RCBP and a **mutein**

ligand with **affinity** for the RCBP; measuring the amount of signal given off by the FlashPlate after allowing for equilibrium; and calculating the relative **affinity** of the **mutein** ligand versus the native ligand. The **IL-4** **mutein** may be used to treat immune disorders, cancers or tumors, abnormal cell growth, allergies or allergic inflammatory diseases, e.g. asthma. (51pp)

L131 ANSWER 42 OF 50 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2001186798 EMBASE
TITLE: Sulfhydryl-2 domain-containing protein tyrosine phosphatase-1 is not a negative regulator of interleukin-4 signaling in murine mast cells.
AUTHOR: White E.D.; Andrews R.P.; Khurana Hershey G.K.
CORPORATE SOURCE: Dr. G.K. Khurana Hershey, Division of Pulmonary Medicine, Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229, United States. Gurjit.Hershey@chmcc.org
SOURCE: Journal of Leukocyte Biology, (2001) 69/5 (825-830). Refs: 52
ISSN: 0741-5400 CODEN: JLBIE7
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Sulfhydryl-2 domain-containing tyrosine phosphatase-1 (SHP-1) has an important role in the negative regulation of many receptors including the interleukin (IL)-4 receptor. Motheaten mice (me/me) have a homozygous mutation in SHP-1 and do not possess functional SHP-1. Pre-B-cell lines derived from me/me mice have been reported to display prolonged IL-4-dependent activation of signal transducer and activator of transcription-6 (Stat6). We evaluated IL-4-dependent Stat6 activation and Fc.epsilon. receptor 1 (Fc.epsilon.RI) modulation in bone marrow-derived mast cells (BMMCs) from me/me and wild-type mice. IL-4 down-regulated Fc.epsilon.RI expression in wild-type BMMCs but had no effect on Fc.epsilon.RI expression in me/me BMMCs. Furthermore, me/me mast cells did not exhibit enhanced or prolonged IL-4-induced Stat6 activation compared with wild-type cells, indicating that mast cells possess alternative tyrosine phosphatases that are responsible for down-regulating Stat6 or can substitute for SHP-1. Thus, SHP-1 is not a negative regulator of IL-4 signaling in BMMCs. These results demonstrate the complexity and cellular **specificity** of these signaling pathways and indicate a previously unrecognized role for SHP-1 in murine mast cells.

L131 ANSWER 43 OF 50 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96272373 EMBASE
DOCUMENT NUMBER: 1996272373
TITLE: Interleukin-4-specific signal transduction events are driven by homotypic interactions of the interleukin-4 receptor .alpha. subunit.
AUTHOR: Lai S.Y.; Molden J.; Liu K.D.; Puck J.M.; White M.D.; Goldsmith M.A.
CORPORATE SOURCE: Gladstone Inst. Virology Immunology, University of California,San Francisco, CA, United States
SOURCE: EMBO Journal, (1996) 15/17 (4506-4514).
ISSN: 0261-4189 CODEN: EMJODG
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Interleukin-4 (IL-4) exerts its effects through a hetero-dimeric receptor complex (IL-4R), which contains the IL-4R.alpha. and .gamma.(c) subunits. IL-4R.alpha. also functions with other partner subunits in several receptor types, including the IL-13 receptor. To examine the roles of the individual subunits within IL-4R complexes, we employed a chimeric system that recapitulates native IL-4R function as verified by the activation of the kinases, JAK1 and JAK3, and induction of STAT-6. When a mutant .gamma.(c), subunit in which the four cytoplasmic tyrosines were converted to phenylalanine was paired with the cytoplasmic domain of the IL-4R.alpha. chain, **specificity** within the JAK-STAT pathway was not altered. Signaling events were examined further in cells expressing the IL-4R.alpha. chimera alone without the .gamma.(c), chimera. Ligand-induced homodimerization of these receptors activated the IL-4 signaling program despite the absence of .gamma.(c), including induction of JAK1 and STAT-6, phosphorylation of the insulin-related substrate 1 and cellular proliferation. Thus, homotypic interactions of the IL-4R.alpha. subunit are sufficient for the initiation and determination of IL-4-specific signaling events, and such interactions may be integral to signaling through IL-4R complexes.

L131 ANSWER 44 OF 50 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96208177 EMBASE
DOCUMENT NUMBER: 1996208177
TITLE: CD40-mediated regulation of interleukin-4 signaling pathways in B lymphocytes.
AUTHOR: Siepmann K.; Wohlleben G.; Gray D.
CORPORATE SOURCE: Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road,London W12 0NN, United Kingdom
SOURCE: European Journal of Immunology, (1996) 26/7 (1544-1552). ISSN: 0014-2980 CODEN: EJIMAF
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The importance of cytokines in controlling immunoglobulin isotype switching is well known. Given the defect in switching to IgG, IgA and IgE isotypes in mice and humans that carry mutations in the CD40 and CD40 ligand genes, we have investigated the role of CD40 ligation in controlling B cell responses to interleukin (IL)-4. We have found that CD40-mediated signals cause a fivefold upregulation of IL-4 receptor (IL-4R) on the B cell surface and that this is associated with a 100-1000-fold increase in the cells' responsiveness to the cytokine. While we found no evidence of increased **affinity** or structural change of the receptor, we do find that prestimulation of B cells with anti-CD40 antibodies brings about several changes in the IL-4 signaling pathways. Subsequent delivery of IL-4 to CD40-prestimulated cells provokes intracellular signals distinct from those induced in resting B cells in response to IL-4. While resting B cells phosphorylate Jak3 kinase shortly after IL-4 activation, cells pre-incubated with anti-CD40 exhibit active dephosphorylation of this molecule and phosphorylation of proteins of around 45 kDa upon addition of IL-4. The common .gamma. chain, Jak3 and Jak1 can all be immunoprecipitated in normal amounts with the IL-4R chain after CD40 prestimulation. We show that the observed dephosphorylation of Jak3 may be due to a stable association with the src-homology protein tyrosine phosphatase SH-PTP2. In contrast, the enzyme appears to be

inactive and to dissociate very quickly from the signaling complex in cells that are stimulated with IL-4 alone.

L131 ANSWER 45 OF 50 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95347417 EMBASE
DOCUMENT NUMBER: 1995347417
TITLE: PTB domains of IRS-1 and Shc have distinct but overlapping binding specificities.
AUTHOR: Wolf G.; Trub T.; Ottinger E.; Groninga L.; Lynch A.; White M.F.; Miyazaki M.; Lee J.; Shoelson S.E.
CORPORATE SOURCE: Joslin Diabetes Center, One Joslin Pl.,Boston, MA 02215, United States
SOURCE: Journal of Biological Chemistry, (1995) 270/46 (27407-27410).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB PTB domains are non-Src homology 2 (SH2) phosphotyrosine binding domains originally described in the receptor tyrosine kinase substrate, Shc. By serial truncation, we show that a 174-residue region of Shc p52 (33-206) has full PTB activity. We also show that a 173-residue region of insulin receptor substrate-1 (IRS-1; residues 144-316) has related PTB activity. In vitro both domains bind directly to activated insulin receptors. Binding is abrogated by substitution of Tyr-960 and selectively inhibited by phosphopeptides containing NPXY sequences. Phosphopeptide assays developed to compare PTB domain specificities show that the Shc PTB domain binds with highest **affinity** to .PSI.XN.beta.1.beta.2pY motifs derived from middle T (mT), TrkA, ErbB4, or epidermal growth factor receptors (.PSI. = hydrophobic, .beta. = .beta.-turn forming); the IRS-1 PTB domain does not bind with this motif. In contrast, both the Shc and IRS-1 PTB domains bind .PSI..PSI..PSI.XXN.beta.1.beta.2pY sequences derived from insulin and interleukin 4 receptors, although specificities vary in detail. Shc and IRS-1 are phosphorylated by distinct but overlapping sets of receptor-linked tyrosine kinases. These differences may be accounted for by the inherent specificities of their respective PTB domains.

L131 ANSWER 46 OF 50 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95164541 EMBASE
DOCUMENT NUMBER: 1995164541
TITLE: The rat interleukin 4 receptor: Coevolution of ligand and receptor.
AUTHOR: Richter G.; Hein G.; Blankenstein T.; Diamantstein T.
CORPORATE SOURCE: New York Hosp., Cornell Medical Ctr., Department of Medicine, Division of Allergy-Immunology, 525 East 68th Street,New York, NY 10021, United States
SOURCE: Cytokine, (1995) 7/3 (237-241).
ISSN: 1043-4666 CODEN: CYTIE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A rat interleukin 4 receptor (IL-4R) cDNA was cloned by polymerase chain reaction (PCR) using RNA of Con A activated T cells and primers deduced from mouse and human IL-4R sequences. Sequence analysis revealed an open

reading frame for a putative membrane protein of 800 amino acids in length. It comprises an overall identity of 52 and 78% to its human and mouse homologues, respectively. The extracellular part of the rat IL-4R contains a number of residues including cysteines and a WSXWS motif typical for the cytokine receptor superfamily. Analysis of amino acid exchanges between rat and mouse IL-4 receptors deciphered for replacement (R) or silent (S) mutations suggested different types of selective pressure acting on the extracellular and intracellular domains. A high R/S value that indicates selective pressure for amino acid exchanges was found for the extracellular domain and a low R/S value for the intracellular part of the IL-4R. Since we previously found a similar high R/S value in the rat IL-4 gene encoding the ligand for the IL-4R, the high amino acid exchange rate can best be explained by coevolution between IL-4 and the ligand binding domain of the IL-4R to improve or retain **affinity**
.

L131 ANSWER 47 OF 50 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-061541 [07] WPIDS
DOC. NO. NON-CPI: N2001-046125
DOC. NO. CPI: C2001-017089
TITLE: Stabilizing cytokine useful for treating or preventing allergy, involves **mutating** its amino acid sequence so as to remove solvent-exposed hydrophobic residues or to stabilize secondary structure elements.
DERWENT CLASS: B04 D16 P14 S03
INVENTOR(S): DOMINGUES, H; OSCHKINAT, H; PETERS, J; SERRANO, L
PATENT ASSIGNEE(S): (FARB) BAYER AG; (EUMO-N) EURO MOLECULAR BIOLOGY LAB
COUNTRY COUNT: 93
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000073460	A2	20001207	(200107)*	EN	59

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000049432 A 20001218 (200118)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000073460	A2	WO 2000-IB769	20000523
AU 2000049432	A	AU 2000-49432	20000523

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000049432	A Based on	WO 200073460

PRIORITY APPLN. INFO: GB 1999-12350 19990526
AB WO 200073460 A UPAB: 20010202
NOVELTY - Stabilizing (M1) a cytokine (I) comprises **mutating** the amino acid sequence of the cytokine so as to remove solvent-exposed hydrophobic residues and/or **mutating** the sequence of (I) so as to stabilize one or more secondary structure elements, so that an intermediate formed during the folding of (I) is destabilized relative to

(I) in its folded state.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a cytokine (I) modified by (M1) ;

(2) human **interleukin-4** (**IL-4**) encoding at positions 68-95 in the full length amino acid sequence (S1) and its functionally equivalent fragments or variants;

(3) a peptide (II) comprising a fragment of (I) or its functional equivalent;

(4) production (M2) of (I) or (II) involves introducing a nucleic acid encoding (I) or (II) into a host cell;

(5) a pharmaceutical composition (III) comprising (I) or (II);

(6) preparation of (III) involves bringing (I) or (II) in association with a carrier;

(7) a diagnostic kit comprising (I) or (II);

(8) a transgenic non-human mammal (IV), carrying a transgene encoding (I) or (II);

(9) production of (IV) involves introducing a DNA encoding (I) or (II) into the embryo of a non-human mammal, preferably a mouse; and

(10) preparation of IL-4R alpha protein involves passing a composition containing IL-4R alpha through an **affinity** column to which a **mutant IL-4** protein is bound, washing the column, and eluting IL-4R alpha from the column.

Ala-Ser-Ala-Ala-Glu-Ala-Asn-Arg-His-Lys-Gln-Leu-Ile-Arg-Phe-Leu-Lys-Arg-Leu-Asp-Arg-Asn-Leu-Trp-Gly-Leu-Ala-Gly (S1).

ACTIVITY - Antiallergic; immunomodulatory.

No supporting data is given.

MECHANISM OF ACTION - None given.

USE - (I) and (II) are useful in the manufacture of a medicament for the treatment or prevention of allergy in a mammal, preferably a human (claimed). (I) is useful for producing antibodies which are useful as diagnostic and therapeutic tools.

ADVANTAGE - (M1) allows the stabilization of (I) such that they may be produced recombinantly at low cost and in large volume.

Dwg.0/11

L131 ANSWER 48 OF 50 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-533012 [48] WPIDS
DOC. NO. CPI: C2000-158859
TITLE: New cytokine-binding domain, and (ant)agonist of the cytokine, useful for preventing or treating a cytokine-related condition, e.g. asthma, leukemia, breast cancer, chronic inflammation, immunosuppression or allergy.
DERWENT CLASS: B04 D16
INVENTOR(S): BAGLEY, C J; LOPEZ, A F; MCKINSTRY, W J; PARKER, M W; ROSSJOHN, J; WOODCOCK, J M
PATENT ASSIGNEE(S): (BAGL-I) BAGLEY C J; (MEDV-N) MEDVET SCI PTY LTD; (SVIN-N) ST VINCENTS INST MEDICAL RES
COUNTRY COUNT: 90
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000047620	A1	20000817	(200048)*	EN	42

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000026488 A 20000829 (200062)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000047620	A1	WO 2000-AU79	20000208
AU 2000026488	A	AU 2000-26488	20000208

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000026488 A	Based on	WO 200047620

PRIORITY APPLN. INFO: AU 1999-264 19990511; AU 1999-8576 19990208; AU 1999-8577 19990209

AB WO 200047620 A UPAB: 20001001

NOVELTY - A cytokine-binding domain or portion that binds to at least one cytokine and is capable of transducing a cytokine signal through a single cytokine **receptor**, is new. The domain comprises a portion of the B'-C' loop of Domain 4 of a beta c chain or analogous structure of a cytokine **receptor**.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of identifying a compound having cytokine agonist or antagonist activity comprising:

(a) subjecting a potential cytokine agonist and/or cytokine antagonist compound to a cytokine binding domain or its portion; and

(b) determining the presence of an agonist or antagonist response of the compound on the activity of a cytokine;

(2) a method of identifying a compound having a cytokine antagonist activity comprising:

(a) subjecting a potential cytokine antagonist to a cytokine binding domain or its portion; and

(b) identifying a compound that has bound to the cytokine-binding domain, where the compound has an antagonist response on the activity of the cytokine;

(3) a cytokine (ant)agonist identified by the methods;

(4) an antibody (or its fragment) to a cytokine binding domain; and

(5) a **mutant** cytokine-binding domain, where a **mutation** is directed to any one of the residues selected from Gln340, Ile338 and Met361, or an equivalent residue of a common signaling unit of a cytokine **receptor**.

ACTIVITY - Immunomodulator; cytostatic; antiallergic; hemostatic. No clinical details given.

MECHANISM OF ACTION - Interleukin (IL)-3 (ant)agonist; IL-5 (ant)agonist; granulocyte-macrophage colony-stimulating factor (GM-CSF) (ant)agonist.

USE - The (ant)agonist and antibody to the cytokine are useful for preventing or treating a cytokine-related condition, e.g. survival or activation of eosinophil function, asthma, leukemia, breast cancer, prostate cancer, small cell lung carcinoma, colon cancer, chronic inflammation including rheumatoid arthritis, immunosuppression, allergy, lymphoma, or cachexia. The antagonist inhibits the binding of IL-5, IL-3 or GM-CSF to the IL-5, IL-3 or GM-CSF **receptors** to treat an allergic inflammation, e.g. asthma. The agonist is administered to prevent or treat hematopoesis, boost immune response, suppress embryonic stem cell differentiation, immunostimulation, antitumor activity, expansion of early hematopoietic cells, anemia, or correct thrombocytopenia (all claimed). Specifically, the antagonists of IL-2R beta / gamma and IL-7 are useful as

immunosuppressants. The agonists of IL-6R are useful as antiinflammatory agents and may be used to inhibit myeloma growth. The antagonists of LIFR and IL-3 are useful for implantation of embryos in utero and treating allergy and follicular B cell, respectively. The antagonists of **IL -4**/IL-13 may be used to inhibit IgE production and may be useful in treating asthma and allergies. Furthermore, the antagonist of the leptin **receptor** (OBR) may be useful in treating cachexia, weight loss in conditions such as AIDS, cancer and parasitic diseases. The agonists agents that bind to LIFR and IL-2R beta may be useful in the suppression of embryonic stem cell differentiation and immunostimulation, respectively. The agonists that bind to IL-4R/IL-13 may also have anti-tumor activity.
Dwg.0/7

L131 ANSWER 49 OF 50 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-207062 [18] WPIDS
DOC. NO. NON-CPI: N1998-164450
DOC. NO. CPI: C1998-065275
TITLE: New isolated interleukin-13 binding protein - used to develop products for therapy e.g. for allergic conditions such as asthma or for diagnosis or detection.
DERWENT CLASS: B04 D16 P14
INVENTOR(S): HILTON, D J; NICOLA, N A; SIMPSON, R J; ZHANG, J
PATENT ASSIGNEE(S): (AMRA-N) AMRAD OPERATIONS PTY LTD
COUNTRY COUNT: 79
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9810638	A1	19980319	(199818)*	EN	69
RW: AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9741049	A	19980402	(199833)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9810638	A1	WO 1997-AU591	19970910
AU 9741049	A	AU 1997-41049	19970910

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9741049	A	Based on	WO 9810638

PRIORITY APPLN. INFO: AU 1997-5374 19970227; AU 1996-2262 19960910

AB WO 9810638 A UPAB: 19980507
An isolated proteinaceous molecule (A) or a recombinant or synthetic form, capable of interacting with interleukin-13 (IL-13) or a related cytokine, with a greater **affinity** than soluble IL-13 **receptor** alpha (IL-13R alpha), is new. Also claimed are: (1) an isolated nucleic acid molecule (I) encoding (A); (2) an expression vector comprising a promoter operably linked to (I); (3) a method of purifying IL-13 binding peptide (IL-13BP) or its derivatives from a biological sample including

body fluid or cell culture medium comprising: (a) contacting the biological sample with immobilised IL-13 or an IL-13/**IL-4** hybrid or a binding derivative to form a complex between the IL-13 and its binding protein; and (b) eluting the IL-13BP or IL-13/**IL-4** from the immobilised IL-13 and collecting the eluted IL-13BP or IL-13/**IL-4**; (4) a peptide (B) having first and second portions (B1 and B2) where one of B1 and B2 is IL-13BP or a functional derivative and the other is IL-4BP or a functional derivative where the polypeptide is capable of modulating biological processes involving IL-13 and/or **IL-4**; (5) an antibody (preferably monoclonal) to (A); and (6) a transgenic animal comprising a **mutation** in at least one allele of the gene encoding IL-13BP.

USE - The IL-13BP and derivatives can be used in the antagonism of at least one IL-13 activity. They can be used for treating IL-13 mediated conditions such as certain allergic conditions such as asthma or to inactivate locally administered IL-13 after IL-13 treatment. The products can also be used as diagnostic agents, e.g. for detecting autoimmune diseases. The antibodies can also be used for immunotherapy and may also be used as a diagnostic tool for assessing e.g. apoptosis or monitoring the programme of a therapeutic regimen.

Dwg.0/3

L131 ANSWER 50 OF 50 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1996-050658 [06] WPIDS
DOC. NO. CPI: C1996-016595
TITLE: New human **interleukin-4** **mutants** as antagonists or partial agonists - e.g. for treating allergies, inhibiting transplant rejection etc., having increased in vivo half life or are easier to produce and purify.
DERWENT CLASS: B04
INVENTOR(S): APELER, H; BEUNINK, J; DORSCHUG, M; HANKO, R; HORLEIN, H; SEBALD, W; WEHLMANN, H; WILD, H; DOERSCHUG, M; HOERLEIN, H; DOERSCHUNG, M; HOERLEIN, D; HEINER APELER, J B
PATENT ASSIGNEE(S): (FARB) BAYER AG
COUNTRY COUNT: 53
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
DE 4423131	A1	19960104	(199606)*		15
WO 9601274	A1	19960118	(199609)	GE	38
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE					
W: AU BB BG BR BY CA CN CZ EE FI HU JP KP KR KZ LK LT LV MG MN MW MX NO NZ PL RO RU SD SI SK UA US UZ VN					
AU 9528852	A	19960125	(199618)		
ZA 9505443	A	19960424	(199622)		37
NO 9605621	A	19961230	(199714)		
EP 769020	A1	19970423	(199721)	GE	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
CZ 9603848	A3	19970611	(199730)		
SK 9601692	A3	19970806	(199740)		
JP 10502360	W	19980303	(199819)		38
KR 97703990	A	19970809	(199836)		
HU 77577	T	19980628	(199840)		
AU 705745	B	19990603	(199933)		
EP 769020	B1	20000301	(200016)	GE	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL SE					
DE 59507920	G	20000406	(200024)		
ES 2145280	T3	20000701	(200036)		
US 6130318	A	20001010	(200052)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
DE 4423131	A1	DE 1994-4423131	19940701
WO 9601274	A1	WO 1995-EP2358	19950619
AU 9528852	A	AU 1995-28852	19950619
ZA 9505443	A	ZA 1995-5443	19950630
NO 9605621	A	WO 1995-EP2358	19950619
		NO 1996-5621	19961230
EP 769020	A1	EP 1995-924273	19950619
		WO 1995-EP2358	19950619
CZ 9603848	A3	WO 1995-EP2358	19950619
		CZ 1996-3848	19950619
SK 9601692	A3	WO 1995-EP2358	19950619
		SK 1996-1692	19950619
JP 10502360	W	WO 1995-EP2358	19950619
		JP 1996-503644	19950619
KR 97703990	A	WO 1995-EP2358	19950619
		KR 1996-707590	19961231
HU 77577	T	WO 1995-EP2358	19950619
		HU 1996-3563	19950619
AU 705745	B	AU 1995-28852	19950619
EP 769020	B1	EP 1995-924273	19950619
		WO 1995-EP2358	19950619
DE 59507920	G	DE 1995-507920	19950619
		EP 1995-924273	19950619
		WO 1995-EP2358	19950619
ES 2145280	T3	EP 1995-924273	19950619
US 6130318	A	WO 1995-EP2358	19950619
		US 1996-765012	19961219

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9528852	A	Based on	WO 9601274
EP 769020	A1	Based on	WO 9601274
CZ 9603848	A3	Based on	WO 9601274
JP 10502360	W	Based on	WO 9601274
KR 97703990	A	Based on	WO 9601274
HU 77577	T	Based on	WO 9601274
AU 705745	B	Previous Publ.	AU 9528852
		Based on	WO 9601274
EP 769020	B1	Based on	WO 9601274
DE 59507920	G	Based on	EP 769020
		Based on	WO 9601274
ES 2145280	T3	Based on	EP 769020
US 6130318	A	Based on	WO 9601274

PRIORITY APPLN. INFO: DE 1994-4423131 19940701

AB DE 4423131 A UPAB: 19981021
Human **interleukin-4 mutant** proteins (A), as antagonists or partial agonists of human **IL-4**, are characterised by substitutions at one or more of positions 120-128 and by at least one of the following: (a) N- and/or C- terminal modifications; (b) deletion of potential sites of glycosylation and (c) coupling to a non-protein polymer (I).

USE - (A) are used therapeutically in cases of defective control of immune reactions or autoimmune diseases. Partic. applications are in

treatment/prevention of allergies; transplant rejection; leukaemia and solid tumours that express the **IL-4 receptor** ; excessive prodn. of thrombocytes; coagulation disorders; disorders of lipid or carbohydrate metabolism, also to improve immune status in patients with sepsis. (A) can also be used to raise antibodies that are useful as assay standards or reagents, and in **affinity** purificn.

ADVANTAGE - The additional modifications extend the biological half life of the proteins or simplify their prepn. or purificn. Since (A) are readily soluble in water, they can be admin. systemically or locally, e.g. as a spray for inhalation, or in a depot formulation.

Dwg.0/1

FILE 'HOME' ENTERED AT 14:33:59 ON 04 SEP 2001